



Asthma and atopic dermatitis are associated with increased risk of clinical Plasmodium falciparum malaria

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Asthma and atopic dermatitis are associated with increased risk of clinical *Plasmodium falciparum* malaria

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Article summary

Article focus

- Genetic studies suggest a link between susceptibility to allergy and malaria in Africa
- We hypothesize that atopy increases susceptibility to malaria

Key messages

- Results demonstrate an association between asthma, atopic dermatitis and susceptibility to clinical *P. falciparum* episodes.
- Genetic pre-disposition to asthma or atopic dermatitis impairs the acquisition of clinical immunity to malaria.
- Administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Strengths and limitations

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association.

Abstract

Objectives: To assess the impact of atopy and allergy on the risk of clinical malaria.

Design: A clinical and immunological allergy cross-sectional survey in a birth cohort of 175 children from 1 month to 14 years of age followed for up to 15 years in a longitudinal open cohort study of malaria in Senegal. Malaria incidence data were available for 143 of these children (aged 4 months to 14 years of age) for up to 15 years. Mixed model regression analysis was used to determine the impact of allergy status on malaria incidence, adjusting for age, gender, sickle cell trait and force of infection.

Main outcome measures: Asthma, allergic rhinoconjunctivitis and atopic dermatitis status, the number of clinical *Plasmodium falciparum* malaria episodes since birth and associated parasite density.

Results: Twelve percent of the children were classified as asthmatic and ten percent as having atopic dermatitis. These groups had respectively a two-fold (OR 2.12 95% confidence intervals 1.46 to 3.08; $P= 8 \times 10^{-5}$) and three-fold (OR 3.15, 1.56 to 6.33; $P= 1.3 \times 10^{-3}$) increase in the risk of clinical *P. falciparum* malaria once older than the age of peak incidence of clinical malaria (3 to 4 years of age). They also presented with higher *P. falciparum* parasite densities (Asthma: mean 105.3 parasites/ μ L \pm SE 41.0 vs. 51.3 \pm 9.7; $P= 6.2 \times 10^{-3}$; Atopic dermatitis: 135.4 \pm 70.7 vs. 52.3 \pm 11.0; $P=0.014$). There was no effect of allergy on the number of non-malaria clinical presentations. Individuals with allergic rhinoconjunctivitis did not have an increased risk of clinical malaria nor any difference in parasite densities.

Conclusion: These results demonstrate that asthma and atopic dermatitis delay the development of clinical immunity to *P. falciparum*. Despite the encouraging decrease in malaria incidence rates in Africa, a significant concern is the extent to which the increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines, administration to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Introduction

The World Allergy Organization estimates that 40% of the world's population is concerned by allergic diseases.¹ In developing countries where *Plasmodium falciparum* malaria is endemic, prevalence of allergy is significantly lower, but is on the increase.² T helper type 2 (Th2) cells, their related cytokines, IgE, eosinophils and mast cells play a major role in allergic inflammation. Orientation of the immune response towards a Th1 profile is crucial for immunity to intracellular pathogens,³ whereas orientation towards a Th2 profile drives immunity to extracellular pathogens and antigens resulting in class switching giving rise to IgE-producing B cells.⁴ A role of the Th1/Th2 balance in the development of clinical malaria following infection by *P. falciparum* has been suggested by numerous studies.⁵⁻⁷ Whilst it is recognised that acquired anti-parasite immunity is IgG dependent,⁸ parasite-specific IgE also impact upon the clinical outcome of infection. For example, higher IgE but not IgG levels have been observed in patients with cerebral malaria than those with uncomplicated *P. falciparum* infection.⁹ The role of IgE, however, remains unclear.¹⁰

The interplay between infectious agents and allergy is ambiguous. On the one hand, for example, severe respiratory syncytial virus infection in infants increased the risk of allergic rhinoconjunctivitis and allergic asthma.^{11,12} On the other hand, measles,¹³ hepatitis A¹⁴ and tuberculosis¹⁵ seemingly reduce atopy. Although, an atopic condition can increase incidence of disease, such as the case for the skin commensal *Staphylococcus aureus* in patients with atopic dermatitis,¹⁶ an atopic tendency *per se* does not generally lead to increased illness from infectious agents.

Genome wide studies have identified chromosomal regions linked to clinical malaria, all of which overlap with those previously identified to be involved in atopic dermatitis, asthma, atopy and IgE levels,¹⁷⁻¹⁹ suggesting that common mechanisms may be involved in both pathologies.²⁰ Chromosomal region 5q31 that has been repeatedly shown to be associated with control of parasite density and contains a cluster of cytokines, among which IL12B has been previously associated with psoriasis.²¹ The other regions, 13q13-q22, 5p15-p13 and 12q21-q23, contain genes involved in innate immunity, notably the interleukin 7 receptor, and several involved in tumour necrosis factor synthesis [C1q and tumour necrosis factor related protein 3 (C1QTNF3)] and a gene involved in the complement system (C9).²⁰

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3 Several additional lines of evidence support the concept that susceptibility to malaria and
4 atopy may be related to similar immunological defects. In Ethiopia, a history of malaria was
5 associated with atopy.²² A mouse model for human atopic disease was found to be very
6 susceptible to murine malaria and a major locus for atopic disease mapped close to the
7 region controlling parasite density.²³ This region contains several candidate genes that have
8 effects on T-cell function.²³
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14 Moreover, a direct effect of histamine in the malaria pathogenesis has been found using
15 genetic and pharmacological approaches²⁴ and increased levels of histamine are associated
16 with the severity of disease in humans infected with *P. falciparum* and in animal malaria
17 models.^{25,26}
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22 To test the hypothesis that allergy impacts upon clinical *P. falciparum* malaria, we performed
23 a clinical allergy cross-sectional study in the family-based longitudinal cohort from Senegal
24 previously used for the genome linkage study²⁰ and analysed the impact of asthma, atopic
25 dermatitis, allergic rhinoconjunctivitis on the incidence of clinical *P. falciparum* episodes and
26 the maximum parasite density during each episode.
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33 **Methods**

34 **Population and outcome data**

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38 The malaria research program conducted in Dielmo village in Senegal has been ongoing
39 since 1990 as described elsewhere.²⁷ In brief, between 1990 and 2008, a longitudinal study
40 involving the inhabitants of the village of Dielmo, Senegal, was carried out to identify all
41 episodes of fever. The study design included daily medical surveillance with systematic blood
42 testing of individuals with fever and examination of 200 oil-immersion fields on a thick blood
43 film for malaria parasites (about 0.5 μ L of blood). Each individual was given a unique
44 identification code and details of family ties, occupation, and precise place of residence were
45 recorded on detailed maps of each household with the location of each bedroom. All
46 households were visited daily, absenteeism recorded, and the presence of fever or other
47 symptoms assessed. We systematically recorded body temperature at home three times a
48 week (every second day) in children younger than 5 years, and in older children and adults in
49 cases of suspected fever or fever-related symptoms. In cases of fever or other symptoms,
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3 blood testing was done at the dispensary by finger prick, and we provided detailed medical
4 examination and specific treatment. Parasitologically confirmed clinical malaria episodes
5 were treated according to national guidelines. From 1990 to 2008, four different drug
6 regimens were implemented: Quinine from 1990 to 1994, Chloroquine from 1995 to 2003,
7 Fansidar (sulfadoxine-pyrimethamine) from 2004 to mid-2006 and Artemisinin-based
8 combination therapy (ACT; Amodiaquine- sulfadoxine-pyrimethamine) from mid-2006 to
9 2008.

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11 Parasite positivity was established as follows. Thick blood films were prepared and stained
12 by 3% Giemsa stain. Blood films were examined under an oil immersion objective at x1000
13 magnification by the trained laboratory technicians and 200 thick film fields were examined
14 to count the number of asexual and gametocyte parasite stages. Asexual parasite densities
15 (per μL) were calculated by establishing the ratio of parasites to white blood cells and then
16 multiplying the parasite count by 8,000, the average white blood cell count per μL of blood.

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18 Malaria transmission in Dielmo is intense and perennial. We conducted a cross-sectional
19 survey to estimate the prevalence of symptoms related to allergic diseases among 175
20 children aged from 1 month to 14 years old who were born during the malaria research
21 program.

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23 Both the longitudinal and cross-sectional surveys were approved by the Ministry of Health of
24 Senegal. Informed consent of the volunteers is renewed every year. More specifically for the
25 cross-sectional survey, after informing about the procedures and the purpose of the study,
26 written informed consent was obtained from parents or guardians of children either by
27 signature or by thumbprint on a voluntary consent form written in both French and Wolof,
28 the main local language. Consent was obtained in the presence of the school director, an
29 independent witness.

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31 The family structure (pedigree) was available after a demographic census performed for
32 every volunteer at his adhesion in the project. A verbal interview of mothers or key
33 representatives of the household was used to obtain information on genetic relationships
34 between studied individuals, their children, their parents, and to identify genetic links
35 among the population. The total pedigree comprised 828 individuals, including absent or
36 dead relatives, composed of ten independent families that can be sub-divided into 206
37 nuclear families (father – mother couples with at least one child) with an average of 3.6
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3 children each. Genetically related nuclear families occur because of multiple marriages and
4 marriages among related individuals. Previous typing with microsatellites has enabled the
5 construction of a pedigree based on Identity-by-Descent using MERLIN.^{20,28} The mean
6 coefficient of inbreeding is 0.0008. Newborns since this original genetic analysis were added
7 to the family of the parents in question. The 143 children, with both allergy and malaria
8 data, belonged to 61 nuclear families and comprised 30 singletons, 102 siblings and 11 half-
9 sibs (yielding 55 half-sib pairs). The mean genetic relatedness (by pedigree) of the 143
10 children is 0.0114 (range: 0.0013 to 0.022).

17 *P. falciparum clinical episodes*

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20 *P. falciparum* malaria clinical episode phenotypes analysed were: (i) clinical *P. falciparum*
21 infections treated with anti-malarial therapy and (ii) the highest parasite density during the
22 *P. falciparum* clinical episode. A clinical *P. falciparum* episode was defined as a clinical
23 presentation with fever (axillary temperature $\geq 37.5^{\circ}\text{C}$) and/or other clinical signs suggestive
24 of malaria associated with a thick blood smear positive for *P. falciparum* and that was
25 treated with anti-malarial therapy. Repeated clinical malaria presentations within 15
26 consecutive days were not considered to be independent and were excluded from the
27 analyses, unless there was a negative thick blood smear between two clinical presentations.
28 We also excluded observations in any trimester for which the individual was not present for
29 at least one third of the time.

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31 We calculated the quarterly incidence rate of clinical *P. falciparum* episodes in children
32 below the age of 15 years as the ratio of the total number of clinical *P. falciparum* episodes
33 during the trimester divided by the total number of person-trimesters surveyed. Incidence
34 rate is expressed as cases per 100 person-trimesters (see Supplementary Figure S1). This
35 rate was used in the analysis to approximate the force of infection (exposure level) within
36 the targeted population at the time of a given clinical *P. falciparum* episode.

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38 The total number of clinical presentations per trimester that were not attributable to *P.*
39 *falciparum* was tabulated. Repeated non-malaria presentations within seven consecutive
40 days were not considered to be independent and were excluded.

41 *Allergic diseases and atopic status*

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3 The International Study of Asthma and Allergies in Childhood (ISAAC) diagnostic criteria have
4 been shown to be reproducible, adequate and able to discriminate children with allergic
5 diseases in different areas of the world.² The standardized ISAAC questionnaire originally
6 written in English was translated into French in compliance with ISAAC guidelines²⁹, adapting
7 it to the usual local customs following advice from local clinicians and paediatric
8 allergologists (Acknowledgements and Technical Appendix). The adequacy and reliability of
9 the translated questionnaire had been previously confirmed by a pilot study on 30 randomly
10 selected children in the same community. The questionnaire was completed by specially
11 trained health workers during an oral interview conducted in Wolof with children and their
12 mothers or guardians.
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21 To assess the prevalence of allergic diseases in children, we used the positive and negative
22 predictive values of the ISAAC questionnaire diagnosis criteria developed for subtropical
23 countries.³⁰ Each question was scored according to the medical diagnosis of paediatricians
24 and paediatric allergologists. Positive or negative answers were thus graded on the basis of
25 symptom sensitivity, specificity, frequency, location or early onset. For each allergic disease,
26 three categories of symptom severity, *severe*, *moderate*, and *none*, were defined as follows:
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32 *Asthma – severe* symptoms if the child had “wheezing or whistling in the chest before the
33 age of two years” and “more than three times” or severe enough to “limit his/her speech”;
34 *moderate* symptoms if the child had “wheezing or whistling in the chest before the age of
35 two years” and “in the past 12 months”; and *none* otherwise.
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41 *Allergic rhinoconjunctivitis – severe* symptoms if the child had “sneezing, runny or stuffy nose
42 in the past 12 months” and “more than five times a year”, and “itchy, watery eyes or tropical
43 endemic limboconjunctivitis (TELC) in the past 12 months”; *moderate* symptoms if the child
44 had “sneezing, runny or stuffy nose in the past 12 months”, and “itchy, watery eyes or TELC
45 in the past 12 months”; and *none* otherwise.
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50 *Atopic dermatitis – severe* symptoms if the child had “scaly or exudating, crusted and pruritic
51 patches in the past 12 months” and “affecting any of the following characteristic areas: face,
52 around the ears or eyes, folds of armpits or elbows or groin, behind the knees, under the
53 buttocks”, and “onset of symptoms before the age of two years”; *moderate* symptoms if the
54 child had “scaly or exudating, crusted and pruritic patches in the past 12 months” and
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3 “affecting any of characteristic areas (see above)”, and “onset of symptoms before the age
4 of four years”; and *none* otherwise.

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7 The inter-relationships between variables reflecting the severity of symptoms of the three
8 allergic diseases were used to identify children at high risk of atopy. The *high probability*
9 group was defined by the prevalence of at least one of any *severe* symptoms or two of any
10 *moderate* symptoms. The *probable* group was defined as those with *moderate* symptoms
11 from one of the three allergic diseases and remaining children were classified in the *unlikely*
12 group.

13 14 15 16 17 18 *Helminths*

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20 Helminthic infections are common in this region and are known to modify the clinical course
21 and outcome of both allergic diseases and malaria.^{31,32} We therefore carried out a helminth
22 survey for 91 individuals present during the cross-sectional survey. Diagnosis was performed
23 by stool examination by microscope and by the Kato technique to search for the presence of
24 *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*),
25 whipworm (*Trichuris trichiuria*), *Schistosoma mansoni*, and *Strongyloides stercoralis*.
26 Examination for pinworms (*Enterobius vermicularis*) was performed by the anal scotch-test.
27 An anti-helminthic treatment was proposed for all infested individuals.

28 29 30 31 32 33 34 35 *Immunoglobulin E titres*

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37 Specific IgE titres were measured by ELISA as previously described.³³ A panel of allergens of
38 potential pertinence to the three classes of allergy was used: (i) Salivary gland extracts (SGE)
39 of two mosquito species present in the study cohorts, *Aedes aegypti* and *Anopheles gambiae*
40 *sensu stricto*, and (ii) *P. falciparum* parasite extract were prepared as previously described³¹;
41 (iii) House dust mite spp. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*;
42 (iv) a mix of pollen allergens from five ubiquitous gramineae spp. [Cock’s-foot (*Dactylis*
43 *glomerata*), Timothy grass (*Phleum pratense*), Sweet Vernal grass (*Anthoxanthum*
44 *odoratum*), Perennial ryegrass (*Lolium perenne*), Kentucky Bluegrass (*Poa pratensis*)] (all
45 from Stallergenes, France).

46 47 48 49 50 51 52 53 **Statistical analysis**

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55 Statistical analyses were performed using R version 2.12.0 (The R Foundation for Statistical
56 Computing, Vienna, Austria). To address the effect of allergic status on the risk of clinical *P.*
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3 *falciparum* episodes, we performed Generalized Linear Mixed Models (GLMM) extended to
4 pedigree data using the *pedigreemm* package for R to account for the non-independence of
5 individuals because of family relationships, shared house and for repeated measures from
6 the same individual (Technical Appendix). Correlated individual effects due to familial
7 relationships were taken into account by using the pedigree-based genetic relatedness
8 matrix that contains the genetic covariance among all pairs of individuals in the study cohort
9 and is calculated using the pedigree information.³⁴ Shared house and repeated measures
10 from the same individual were modelled as random effects. All random effects were
11 assumed to be normally distributed, and conditional on these random effects, the
12 dependent variable had: (i) a Binomial distribution when the studied phenotype was the
13 occurrence of a clinical *P. falciparum* episode treated with anti-malarial therapy during a
14 trimester, (ii) a Gaussian distribution when the studied phenotype was the logarithm of the
15 maximum parasite density during a given clinical *P. falciparum* episode, and (iii) a Poisson
16 distribution when the studied phenotype was the number of non-malaria episodes per
17 trimester. The effects of allergy disease classes on these dependent variables were modelled
18 as fixed effects. Allergy classes were reduced to two levels, *Severe* or *moderate* vs. *none* for
19 analyses of asthma, atopic dermatitis and allergic rhinoconjunctivitis and *high probability* vs.
20 *probable* and *unlikely* for atopic tendency. Co-variables included sickle cell trait³³, gender,
21 number of days present on site during the trimester, trimestrial incidence of *P. falciparum*
22 and age. Age was initially analysed as a continuous covariate. To assess the age-specific
23 effect of allergy, age was categorised into two levels (<3.5 years of age and ≥3.5 years of
24 age, based on the age of peak clinical incidence) and allergy class was nested within age
25 class. The age threshold was varied from 1.5 years to 5.5 years of age and the data re-
26 analysed to assess at which age there was the strongest effect. The association of allergy
27 classes with IgE levels was analysed by box-cox transforming the data and fitting a GLMM
28 with a normal distribution.

51 Results

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54 Of the 205 eligible children aged under 15 years involved in the family-based longitudinal
55 study, 175 (85.4 %) participated in the cross-sectional survey to assess the prevalence of
56 related symptoms of allergic diseases. All eligible children present at the time of the survey
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3 were included; no explicit refusal to participate was recorded. The study cohort was aged
4 from 1 month to 14 years 11 months. The sex-ratio (male/female) was 0.94.

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7 From 1994 until 2008, 143 of the children participating in the cross-sectional survey were
8 present for at least 31 days in any trimester during the study period generating a total of
9 3,093 person-trimesters of presence (Supplementary Table S1). There were 2,065 treated *P.*
10 *falciparum* clinical episodes (per individual: median 11, range 0-47)(Supplementary Table
11 S2). The age peak of incidence of *P. falciparum* episodes occurred at 3 to 4 years of age
12 (Figure 1). There were 1,868 non-malaria episodes (median 12, range 0-37) (Table S2). These
13 non-malaria clinical presentations were associated with headache (38 %), chills (32 %), cough
14 (13 %), vomiting (11 %) and diarrhoea (6 %).

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17 The prevalence of moderate or severe asthma symptoms was respectively 2.3 % and 10.3 %
18 (Table 1). The prevalence of moderate or severe allergic rhinoconjunctivitis symptoms was
19 respectively 6.3 % and 10.3 %. The prevalence of moderate or severe atopic dermatitis
20 symptoms was respectively 6.3 % and 2.9 %. On the basis of symptom severity, an atopic
21 tendency was estimated to be unlikely for 68.0 %, probable for 9.1 % and highly probable for
22 22.9 % of the 175 children. The frequency of each allergy class in children for whom malaria
23 data were available is shown in Table S1.

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26 The risk of treated clinical *P. falciparum* infections was higher for children with high
27 probability of atopy (OR 1.65, 95% confidence intervals 1.20 to 2.26; $P=0.002$) (Table 2), after
28 adjusting for age, sickle cell trait and the exposure level. Gender was not found to be
29 significant. Analysing the impact of atopy in children younger and older than the peak age of
30 clinical incidence (3 to 4 years old), revealed that atopy increased the risk of *P. falciparum*
31 episodes in children at an age greater than 3.5 years (OR 2.02, 1.39 to 2.93; $P=2 \times 10^{-4}$), but
32 not in children of age prior to the peak clinical incidence (OR 1.38, 0.92 to 2.08; $P=0.124$)
33 (Table 2). This increased risk resulted in an ever increasing cumulative number of *P.*
34 *falciparum* episodes with age beyond that of peak clinical incidence (Figure 2. See
35 supplementary Figure S2 for model predictions for comparison).

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38 Analysis by allergy category revealed that asthma (severe or moderate) increases the risk of
39 *P. falciparum* episodes (OR 2.12, 1.46 to 3.08; $P= 8 \times 10^{-5}$) and this again only in children of
40 age greater than 3.5 years old (OR 2.33, 1.50 to 3.61; $P= 1.5 \times 10^{-4}$). Atopic dermatitis
41 increased the risk of clinical malaria in children older (OR 3.15, 1.56 to 6.33; $P= 1.3 \times 10^{-3}$) but
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3 not younger than 3.5 years of age (Table 2). Allergic rhinoconjunctivitis was not associated
4 with increased risk of clinical malaria at any age (Table 2). The impact of atopy, asthma and
5 atopic dermatitis can be clearly seen in the ever-increasing number of cumulative *P.*
6 *falciparum* episodes beyond the age of the onset of clinical immunity in the population, 3.5
7 years of age (Figure 2). There is no difference in the number of clinical malaria episodes prior
8 to this age in individuals with or without an allergic condition. Analysis using different age
9 thresholds (from 1.5 to 5.5 years of age) revealed similar OR for thresholds of 2.5, 3.5 and
10 4.5 years of age. The maximum OR for increased malaria occurred in children older than 4.5
11 years of age and with atopy or atopic dermatitis, whereas for the asthma group it occurred
12 in children after 3.5 years of age (Supplementary Table S3).

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21 There was no impact of any allergic disease on the number of non-malaria episodes by
22 trimester (Supplementary Table S4).

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25 The impact of atopy, asthma and atopic dermatitis on the maximum *P. falciparum* parasite
26 density during a given clinical malaria episode mirrored that of the risk of *P. falciparum*
27 episodes. Parasite density was significantly higher for children with allergic disease older
28 than 3.5 years of age (Table 3 and supplementary Figure S3 for residuals of the fitted model).
29 As the log-transformed data were left skewed, we additionally analysed using box-cox
30 transformation and probit normalization of the data. The results were qualitatively the same
31 (Supplementary text and Figures S4-S8). Allergic rhinoconjunctivitis had no impact on the
32 parasite density (Table 3). Analysis using different age thresholds yielded similar qualitative
33 conclusions as seen with the number of clinical episodes (Table S3).

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42 Individuals with moderate or severe symptoms of atopic dermatitis had significantly higher
43 specific IgE titres against *Ae. aegypti* (P=0.004) and *An. gambiae* SGE (P<0.001). There were
44 no detectable specific anti-*P. falciparum* IgE. Individuals with moderate or severe symptoms
45 of allergic rhinoconjunctivitis did not have significantly higher IgE titres against the tested
46 gramineae (P=0.28), although titres decreased with age (P=0.035). There was also no effect of
47 asthma on IgE titres against the house dust mite spp. tested (*D. farinae* P=0.60 & *D.*
48 *pteronysinus* P=0.27).

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Only five individuals were infested with helminths (two *Ancylostoma*, one *Strongyloides*, one
Trichuris and one *Enterobius*).

Discussion

Principal findings

Establishing the allergic status of children up to the age of 15 years old followed for malaria since birth, revealed an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

Strengths and weaknesses of the study

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association. In addition, although allergy diagnosis for children under 2 years of age is not considered reliable, there were only 15 individuals under 2 at the time of the allergy study of the 143 for whom malaria and allergy data were available.

Meaning of the study

Under intense malaria transmission, after repeated exposure to the parasite, children develop a clinical immunity³⁵, whereby they tolerate elevated parasite densities without showing clinical symptoms. In this cohort, the population mean onset of clinical immunity occurred at 3 to 4 years of age. Although clinical immunity is accompanied by a reduction in parasite density, effective anti-parasite immunity develops much more slowly³⁶ with individuals achieving a state of premunition, whereby they maintain low-grade parasite densities in an asymptomatic state.³⁷ We show here that children with clinically defined asthma or atopic dermatitis have an increased risk of presenting with *P. falciparum* malaria episodes requiring treatment once passing the age of peak clinical incidence. They also had higher parasite density during clinical episodes, suggesting a reduced ability to control parasite replication. The observed increase in clinical incidence of malaria in patients with asthma or atopic dermatitis is not likely to be the result of increased frailty of such individuals; these individuals did not come more frequently to the clinic with non-malaria

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3 symptoms. Our previous genome linkage study identifying chromosomal regions²⁰
4 associated with malaria that overlap with those previously shown to be linked to
5 asthma/atopy suggests that there may be a shared genetic basis to these pathologies rather
6 than any causative effect of one on the other. This is consistent with the increased
7 susceptibility to malaria of mouse atopic models.²³
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10 11 12 **Comparison with other studies**

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14 A previous study in Ethiopia (East Africa) found that a history of malaria (yes/no) increased
15 risk of atopic dermatitis in 306 cases compared to 426 controls as characterized using the
16 ISAAC questionnaire.²² The only other epidemiological study that has previously examined
17 the link between malaria and atopy³⁸ also interpreted the result from the perspective of the
18 impact of malaria on atopy. They examined the re-infection rate with *P. falciparum* over a 5-
19 year period in 91 children that were subsequently classified as atopic or not using skin prick
20 tests (SPT) with house dust mite antigen. Their conclusion was that, as with measles¹³ and
21 tuberculosis¹⁵, malaria infection reduces atopy. However, the study lacked previous infection
22 data since birth of the participating individuals and focussed on atopy as determined by SPT
23 against a single allergen. The case-control study of atopic dermatitis risk factors cited above
24 found no overall association between allergen skin sensitization and atopic dermatitis. We
25 also found no evidence of increased IgE titres against house dust mites in the asthmatic or
26 atopic dermatitis groups or against grass pollen in individuals with allergic
27 rhinoconjunctivitis. Such differences likely reflect the different IgE reactivity profiles due to
28 differences in allergen exposure in Africa.³⁹ There was no evidence of anti-parasite IgE in this
29 cohort of children. We previously showed that circulating anti-parasite IgE titres were
30 strongly positively correlated with anti-mosquito saliva IgE, but became undetectable
31 following malaria exposure, potentially being bound to effector cells.³³ Only mosquito saliva,
32 a known major local allergen, induced a specific IgE response at significantly higher titres in
33 individuals with atopic dermatitis.
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50 Although the immune effectors of clinical immunity are still poorly defined, there is strong
51 evidence that acquired anti-parasite immunity is IgG-dependent⁸ and cytophilic
52 immunoglobulins (IgG1 & IgG3), which are capable of eliminating the parasites by
53 opsonisation and/or by Antibody Dependent Cellular Immunity play an important role in
54 premunition.³⁷ The higher parasite density during symptomatic episodes observed in the
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3 asthma group suggests impaired development of acquired immunity. Impaired acquisition of
4 immunity to malaria in children with asthma or atopic dermatitis may stem from their
5 imbalanced Th1/Th2 response. Indeed, an atopic state may generate a tendency to develop
6 a Th2 type immune response to *P. falciparum*. Dendritic cells that are oriented to a Th2
7 phenotype are more susceptible to orient the acquired immune response towards a Th2
8 profile.⁴⁰ Orientation of the immune response towards a Th2 profile by asthma or atopic
9 dermatitis would result in a poor Th1 response (and hence development of protective IgG
10 immunoglobulins), considered to be the dominant arm of the immune response enabling
11 resistance to infectious disease in children.⁴¹

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20 Many studies have revealed an important role of histamine, a key downstream effector
21 molecule in allergic reaction, in the outcome of a malaria parasite infection.^{24-26,42-45}
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23 Moreover, reports indicate that components of the innate immune system, including
24 eosinophils, basophils, and mast cells (MCs), could play important roles in the pathogenesis
25 of malaria.⁴² Increased levels of histamine in plasma and tissue, derived from basophils and
26 MCs, notably following stimulation by IgE through the high affinity receptor FcεR1, are
27 associated with the severity of disease in humans infected with *P. falciparum* and in animal
28 malaria models.^{25,26} Chlorpheniramine, a HR1agonist reversed resistance to chloroquine and
29 amodiaquine both *in vivo* and *in vitro*.⁴³ Moreover, astemizole, another HR1 agonist, was
30 identified as an anti-malarial agent in a clinical drug library screen.⁴⁴ Finally, *P. falciparum*
31 produces translationally controlled tumor protein, which is a homolog of the mammalian
32 histamine-releasing factor that causes histamine release from human basophils.⁴⁵

40 41 **Further research**

42
43 Our results provide the first birth cohort study addressing the link between malaria and
44 allergic diseases. They contribute to a growing body of evidence that the pathologies are
45 related. ISAAC has revealed a steady but significant increase in prevalence rates of asthma
46 and allergic diseases in Africa. Whilst the majority of studies have focused on large cities,
47 there is increasing urbanization throughout Africa, as well as improved access to primary
48 health care in many areas. A key concern for ISAAC is the extent to which such societal
49 evolution will result in an increase in allergic diseases. Increased urbanization in sub-Saharan
50 Africa is changing the epidemiology of malaria and although resulting in a decrease in risk,
51 will result in more severe clinical malaria in older individuals.^{46,47} Moreover, a large
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3 consumption of anti-malarial drugs in the urban areas provides substantial drug pressure
4 fostering, the selection of drug-resistant parasites. Despite the encouraging recent decrease
5 in malaria incidence rates, even in rural areas, an additional significant concern is the extent
6 to which such an increase in allergy will exacerbate the burden of malaria. Given the
7 demonstrated anti-parasitic effect of anti-histamines,⁴⁸ administration of anti-histamines to
8 atopic children will likely reduce the burden of clinical malaria in these children, increase the
9 efficacy of first-line treatment anti-malarials⁴⁹ and alleviate the non-infectious consequences
10 of atopy. Clinical intervention studies should be envisaged.

17 **What is already known on this topic**

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20 There are several reports of the beneficial effects of anti-histamines for malaria
21 chemoprophylaxis^{24-26,48} as well as our previous work²⁰ showing that chromosomal regions
22 associated with malaria are also linked to allergy and atopy.¹⁷⁻¹⁹ There are two
23 epidemiological studies showing opposite effects of malaria on atopy.^{22,38}

27 **What this study adds**

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30 Using a longitudinal malaria study birth cohort, we identified an association of asthma and
31 atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the
32 increase in risk of malaria associated with these allergic conditions occurred only after the
33 peak clinical incidence of disease in the population, suggesting that they delay the
34 development of clinical immunity to malaria.

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16 OMP, AS and RP contributed to the analysis and interpretation of the data. MH, CL, HB, BG,
17 SB, FDS, AF, AT, LB, OMP, SM, AS and RP critically reviewed the report and approved its final
18 version for submission. All authors had full access to all of the data in the study and can take
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20 are guarantors.
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29 www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and
30 declare: no financial relationships with any organisations that might have an interest in the
31 submitted work in the previous three years; no other relationships or activities that could
32 appear to have influenced the submitted work.
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38 Ethical approval: The allergy study was approved by the Senegalese National Ethics
39 committee (2009/N°46). Renewed approval of the longitudinal malaria study was obtained
40 from the same committee (2006/N°969).
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44 Data sharing: The allergy database will be made available on-line. The longitudinal malaria
45 data set will be made available following discussion with the coordinators of the three
46 Institutes that govern the dataset through contact with the corresponding author.
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Table 1 Classification of Asthma, Allergic rhinoconjunctivitis, Atopic dermatitis and overall Atopic status according to ISAAC questionnaire in children aged 0-14 from a malaria birth cohort. N is total number of children examined and n-malaria represents those for whom malaria data were recorded. F is the number of females and M the number of males.

	N (F/M)	%	n-malaria (F/M)
Asthma symptoms			
None	153 (73/80)	87.43	125 (59/66)
Moderate	4 (1/3)	2.29	4 (1/3)
Severe	18 (6/12)	10.29	14 4/10)
Rhinoconjunctivitis symptoms			
None	146 (64/82)	83.43	120 (52/68)
Moderate	11 (8/3)	6.29	9(6/3)
Severe	18 (6/12)	10.29	14 (6/8)
Atopic dermatitis symptoms			
None	159 (75/84)	90.86	128 (60/68)
Moderate	11 (1/10)	6.29	11 (1/10)
Severe	5 (4/1)	2.86	4 (3/1)
Atopic tendency			
Unlikely	119 (56/63)	68.00	97 (46/51)
Probable	16 (8/8)	9.14	14 (6/8)
Highly probable	40 (16/24)	22.86	32 (12/20)

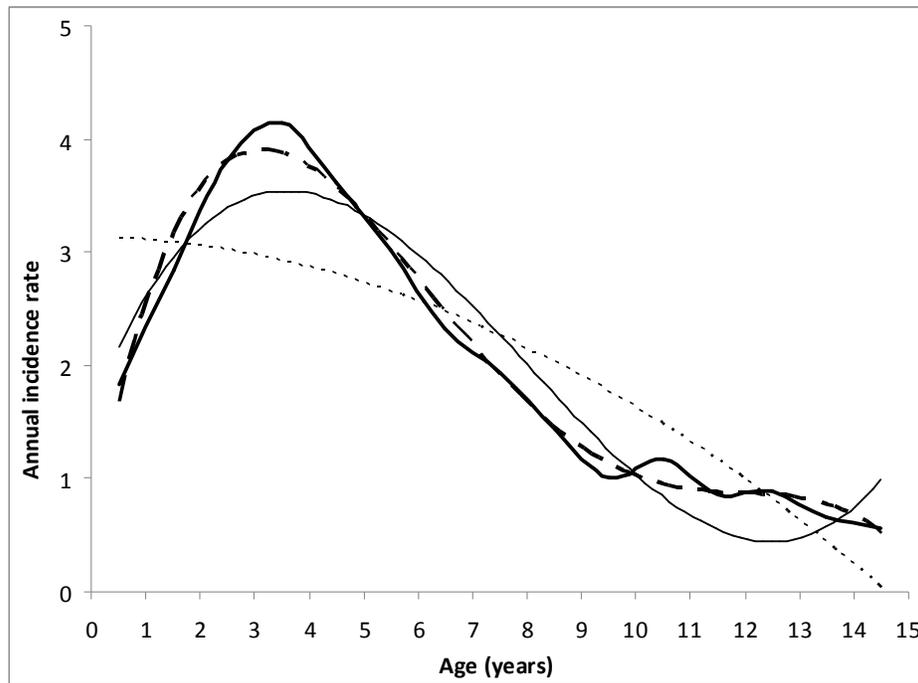
Table 2 Impact of allergy status on risk of *P. falciparum* clinical episodes. Shown are the *P* values and adjusted Odds Ratios with 95% confidence intervals calculated from the mixed model analyses. Values for the covariables Age (≥ 3.5 years of age compared to < 3.5 years of age), Trimestrial incidence of *P. falciparum* clinical episodes and HbAS (beta-globin sickle cell trait; AS compared to AA) are those from the Asthma model analysis. For clarity significant co-variables are shown in bold.

	Age groups < 3.5 years $>$	ORa	95% Confidence Intervals		<i>P</i> value
			Lower	Upper	
Atopy	Both	1.65	1.20	2.26	2.0×10^{-3}
	< 3.5	1.38	0.92	2.08	0.124
	≥ 3.5	2.02	1.39	2.93	2.1×10^{-4}
Asthma	Both	2.12	1.46	3.08	8.0×10^{-5}
	< 3.5	1.50	0.90	2.50	0.122
	≥ 3.5	2.33	1.50	3.61	1.5×10^{-4}
Atopic dermatitis	Both	1.05	0.65	1.70	0.842
	< 3.5	0.84	0.49	1.46	0.539
	≥ 3.5	3.15	1.56	6.33	1.3×10^{-3}
Rhinoconjunctivitis	Both	0.96	0.65	1.41	0.818
	< 3.5	1.05	0.64	1.72	0.853
	≥ 3.5	0.95	0.60	1.52	0.834
Age ≥ 3.5		0.48	0.40	0.57	2.7×10^{-15}
Trimestrial incidence		1.01	1.00	1.01	1.8×10^{-6}
HbAS		0.24	0.12	0.47	3.7×10^{-5}

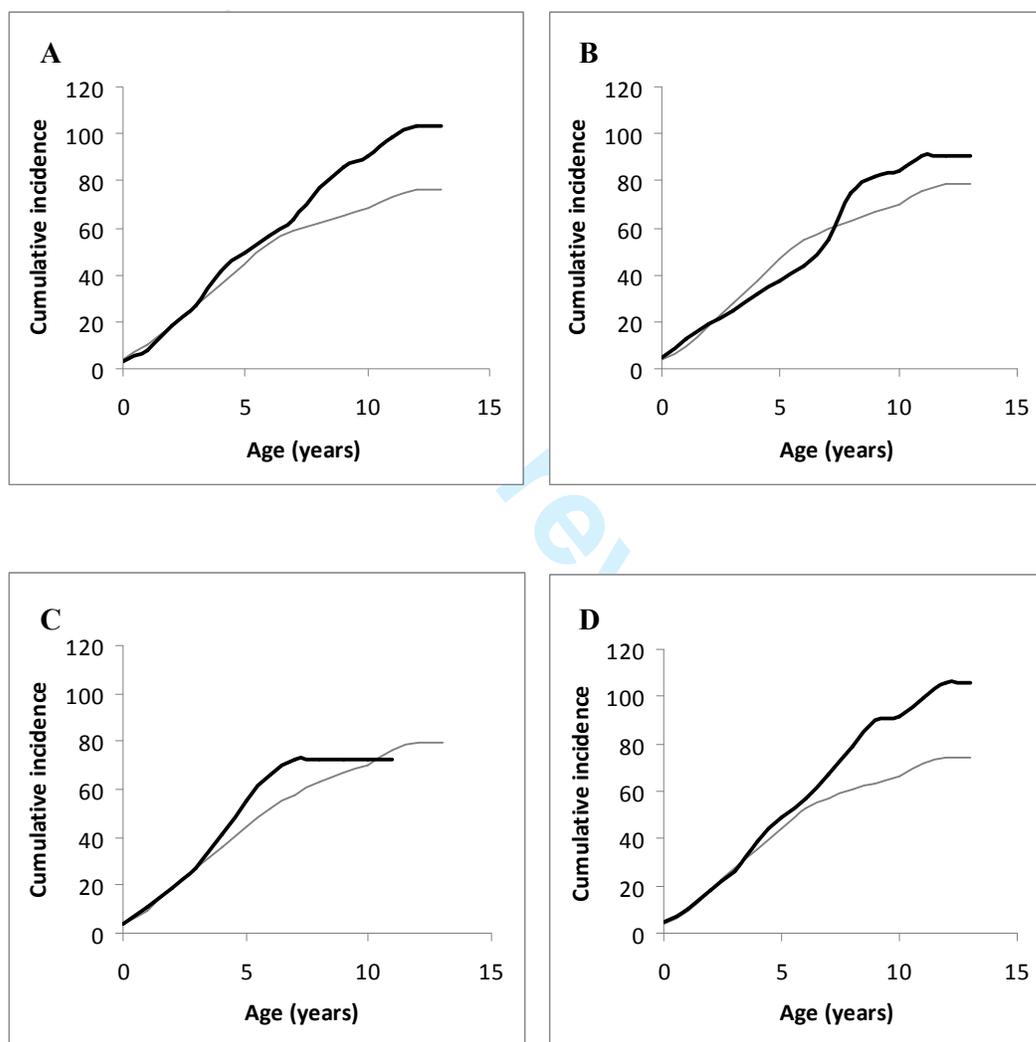
Table 3 Impact of allergy status on the maximum *P. falciparum* parasite density during a clinical malaria episode. Shown are the back-transformed mean parasite densities per microlitre and standard errors (SEM) estimated from the GLMM analyses after taking into account the other co-variables. Significantly different effects are shown in bold for clarity.

Allergic condition	Age groups	Allergic status (No/Yes)	Mean parasite density	SEM	<i>P</i> value	
Atopy	Both	N	76.3	13.8		
		Y	131.0	36.4	0.0158	
	<3.5	N	114.3	23.7		
		Y	171.1	56.0	0.148	
		≥3.5	N	48.4	9.8	
			Y	114.8	37.1	9.5x10⁻⁴
Asthma	Both	N	78.1	14.4		
		Y	148.5	44.3	3.8 x10⁻³	
	<3.5	N	114.8	24.3		
		Y	171.9	74.5	0.167	
		≥3.5	N	51.3	9.7	
			Y	105.3	41.0	6.2 x10⁻³
Atopic dermatitis	Both	N	82.6	15.0		
		Y	93.9	38.9	0.605	
	<3.5	N	122.6	25.5		
		Y	133.9	63.5	0.425	
		≥3.5	N	52.3	11.0	
			Y	135.4	70.7	0.014
Rhinoconjunctivitis	Both	N	81.5	14.8		
		Y	111.4	39.0	0.570	
	<3.5	N	118.8	25.1		
		Y	166.3	69.9	0.537	
		≥3.5	N	54.6	11.3	
			Y	80.9	33.7	0.327

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3 **Figure 1** Annual incidence rate of clinical *P. falciparum* episodes per 100 children (bold
4 line). In order to overcome the fluctuations of the annual incidence rate, we fit second (dotted
5 line), third (dashed line) and fourth (solid line) degree polynomial trend lines to the data (bold
6 line). The corresponding R-squared values are 0.70, 0.91 and 0.99 respectively indicating an
7 accurate fit for third and fourth order polynomials. The inflexion on these two trend lines
8 indicates the onset of acquisition of clinical immunity at approximately 3 to 4 years of age.
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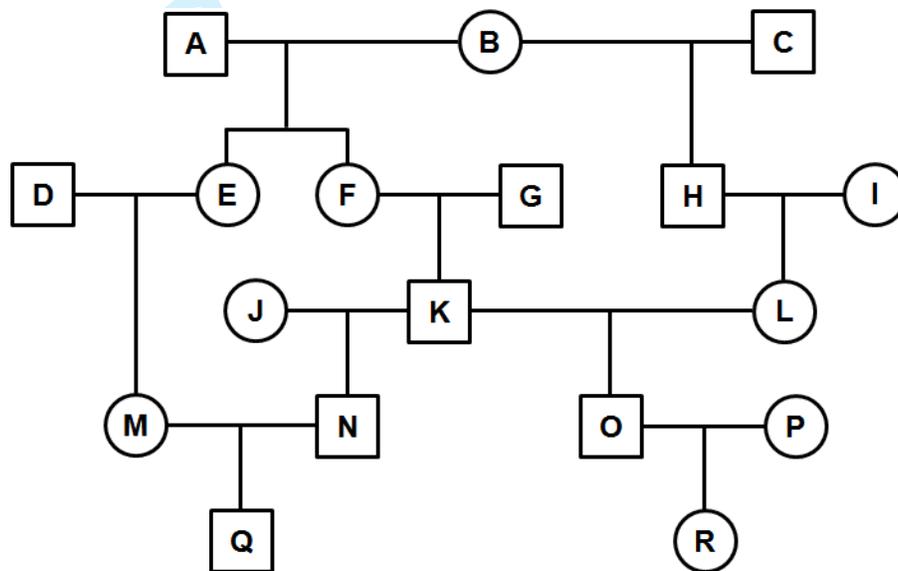
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3 **Figure 2** Mean cumulative number of *P. falciparum* clinical episodes with age for the (A)
4 Asthma, (B) Rhinoconjunctivitis and (C) Atopic dermatitis classes and overall Atopy class
5 (D) (bold lines) compared to individuals without symptoms of each respective allergy type
6 (thin lines). In all cases moderate and severe classes are combined and compared to
7 individuals without allergy symptoms. Note there are no children older than 11 years of age
8 with Atopic dermatitis.
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Pedigree-based genetic relatedness

The Genetic covariance between two individuals can be computed using the pedigree information. For individuals A and B, a given pair in a pedigree, the genetic covariance is computed as $r(A,B) = 2 \times \text{coancestry}(A,B)$ where the *coancestry* between A and B is calculated referring to the method presented by Falconer and Mackay in 1996 (Falconer and Mackay 1996): $\text{coancestry}(A,B) = \sum_p (1/2)^{n(p)} \times (1 + I_{\text{Common Ancestor}})$ where p is the number of paths in the pedigree linking A and B, $n(p)$ the number of individuals (including A and B) for each path p and I_X is the *inbreeding* coefficient of X also equal to the *coancestry* between the two parents of X, I_X is set to 0 if X is a founder.

Illustration: Consider, as an example, the pedigree below containing 18 individuals named {A, B, ..., R} for the calculation of genetic covariance's.



Pedigree structure.

The genetic relatedness between individuals N and O is equal to 0.266. This value is calculated as followed:

The number of paths linking N and O from the pedigree structure above is $p = 2$.

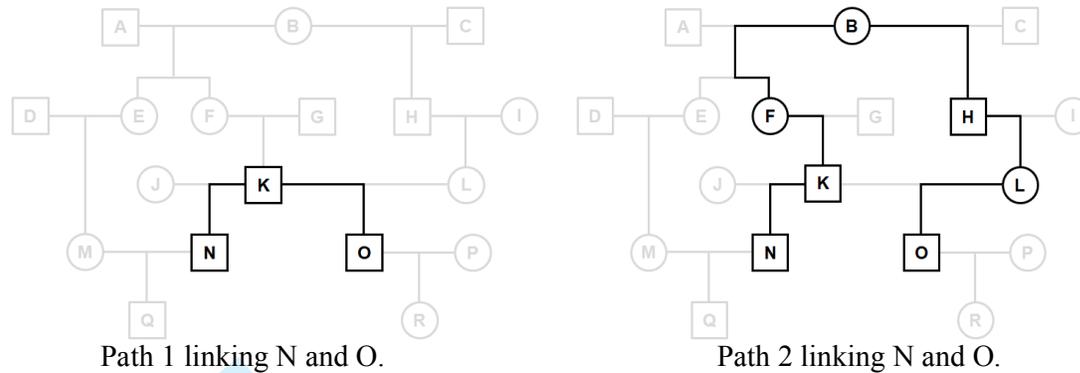
As illustrated below:

- **Path 1** contains $n(1) = 3$ individuals {N, K, O} with K as the common ancestor. Inbreeding coefficient of K, I_K , is the *coancestry* between the two parents of K (F and G) and is null because F and G are not genetically linked.
- **Path 2** contains $n(2) = 7$ individuals {N, K, F, B, H, L, O} with B as the common ancestor. Inbreeding coefficient of B, I_B , is null because B is a founder.

Therefore, genetic relatedness between individuals N and O is:

$$= 2 \times (0.5^{n(1)} \times (1 + I_K) + 0.5^{n(2)} \times (1 + I_B))$$

$$= 2 \times (0.5^3 \times (1 + 0) + 0.5^7 \times (1 + 0)) = 0.266$$



Defining an equivalent model design where individual effects are independent using the genetic relatedness matrix:

Let us rename $Y^* = l(\mu)$. Y^* can be considered as a linearization of the phenotype through the link function l . The expected mean of Y^* and the variance of Y^* are:

- (i) $E(Y^*) = E(X\beta + Z\gamma + \varepsilon)$
 $= E(X\beta) + E(Z\gamma) + E(\varepsilon) = X \times E(\beta) + Z \times E(\gamma) + E(\varepsilon)$
 $= X\beta$ (asymptotically).
- (ii) $\text{Var}(Y^*) = \text{Var}(X\beta + Z\gamma + \varepsilon)$
 $= \text{Var}(Z\gamma + \varepsilon)$ (as $X\beta$ is the fixed part, thus has variance equal to 0)
 $= \text{Var}(Z\gamma) + \text{Var}(\varepsilon)$ (as γ and ε are independent)
 $= Z \times \text{Var}(\gamma) \times Z^T + \text{Var}(\varepsilon)$ (Z^T is the transpose of Z)
 $= Z(A\sigma_g^2)Z^T + I\sigma_r^2$
 $= ZAZ^T\sigma_g^2 + I\sigma_r^2$

If individuals were independent, i.e. $A = I_N$, variance of Y^* could be expressed as $ZZ^T\sigma_g^2 + I\sigma_r^2$. However, using linear algebra theory by the method “Cholesky decomposition of a matrix”, we can show that there is an equivalent expression of the variance of Y^* corresponding to the modeling of data from independent individuals, having γ^* as an equivalent vector of random effects and Z^* an equivalent design matrix relating γ^* to Y^* so that:

$\text{Var}(Y^*) = Z^*(I\sigma_g^2)Z^{*T} + I\sigma_r^2$. $I\sigma_g^2$ is then the covariance matrix of the equivalent independent random individual effects γ^* .

Theorem: Cholesky decomposition of a matrix

If A is a symmetric positive-definite matrix, there is a triangular matrix L so that A can be written as $A = LL^T$. L can be seen as the “square root” of the matrix A .

Note that the genetic relatedness matrix A computed using the pedigree information (Falconer and Mackay 1996) is a positive-definite matrix, unless identical twins are in the pedigree in which case it would be positive semi-definite.

Equivalent model with independent random effects: We set $A = LL^T$ then:

$$\begin{aligned} \text{Var}(Y^*) &= Z(A\sigma_g^2)Z^T + I\sigma_r^2 \\ &= Z(LL^T\sigma_g^2)Z^T + I\sigma_r^2 \end{aligned}$$

$$\begin{aligned}
 &= ZLL^T Z^T \sigma_g^2 + I\sigma_r^2 \\
 &= (ZL)(ZL)^T \sigma_g^2 + I\sigma_r^2 \\
 &= (Z^*)(Z^*)^T \sigma_g^2 + I\sigma_r^2 \quad (\text{where we set } Z^* = ZL)
 \end{aligned}$$

Then, if we define $\gamma^* = L^{-1}\gamma$, we can rewrite the model as:

$$Y^* = X\beta + Z^*\gamma^* + \varepsilon \quad (\text{because } Z\gamma = Z(LL^{-1})\gamma = (ZL)(L^{-1}\gamma) = Z^*\gamma^*),$$

and the γ_i^* are independent, in other terms $\text{Var}(\gamma^*) = I\sigma_g^2$, as demonstrated below:

We assumed that $\gamma \sim N(0, A\sigma_g^2)$. Then $\gamma^* = L^{-1}\gamma$ is also distributed as a multivariate Normal with mean $E(\gamma^*) = L^{-1}E(\gamma) = L^{-1} \times 0 = 0$ and variance:

$$\begin{aligned}
 \text{Var}(\gamma^*) &= (L^{-1}) \times \text{Var}(\gamma) \times (L^{-1})^T \\
 &= (L^{-1}) \times A\sigma_g^2 \times (L^{-1})^T = (L^{-1})LL^T(L^{-1})^T \sigma_g^2 \\
 &= (L^{-1}L)(L^{-1}L)^T \sigma_g^2 \\
 &= I\sigma_g^2
 \end{aligned}$$

The random effects are now independent and then the classical mixed model assuming independence between levels (here individuals) is applied, and the estimate of fixed effects obtained are fine, i.e. corrected for genetic relationships.

References

Falconer DS, Mackay TFC (1996) Introduction to Quantitative Genetics. 4th Edn. London: Longman.

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Supplementary Tables

Table S1 Number of person-trimesters contributed by number of children by age class and the number who had severe/moderate allergy symptoms, for whom malaria data were also available. AS – Asthma, AD – Atopic dermatitis, RC – Rhinoconjunctivitis. Shown also are the numbers of these individuals suffering from two or all three allergy conditions.

Age group	N° person-trimesters	N° people	AS	AD	RC	AS+AD	AS+RC	AD+RC	AS+AD+RC
]1	7	6	1	2	2	0	1	0	0
]2	21	9	0	1	3	0	0	0	0
]3	48	11	1	1	2	0	0	1	0
]4	119	12	1	2	3	0	0	1	0
]5	102	11	3	4	3	2	1	2	1
]6	125	11	1	1	0	0	0	0	0
]7	303	11	1	2	1	1	0	0	0
]8	340	12	1	1	1	1	0	0	0
]9	362	10	2	0	1	0	1	0	0
]10	610	17	1	0	3	0	0	0	0
]11	77	4	2	1	0	0	0	0	0
]12	484	16	3	0	3	0	1	0	0
]13	390	10	1	0	0	0	0	0	0
]14	105	3	0	0	1	0	0	0	0
Total	3093	143	18	15	23	4	4	4	1

Table S2 Summary of total number of person-trimesters with non-malaria and symptomatic *P. falciparum* clinical presentations and total number of non-malaria episodes according to age class. Given are the number of people contributing to each type of presentation.

	Age group (years)	
	<3·5	≥3·5
Total person-trimesters	1283	1810
People	126	113
Total <i>P. falciparum</i> symptomatic trimesters	963	1102
People	114	108
Total non-malaria episodes	754	1114
People	123	109

Table S3 Effect of changing age threshold on impact of allergy on the risk of clinical malaria and concomitant parasite density. Given are Odds Ratio with 95% confidence intervals, for clinical malaria episodes and the beta coefficient and standard error for parasite density. Corresponding P values are also given. Values are from the nested GLMM analyses.

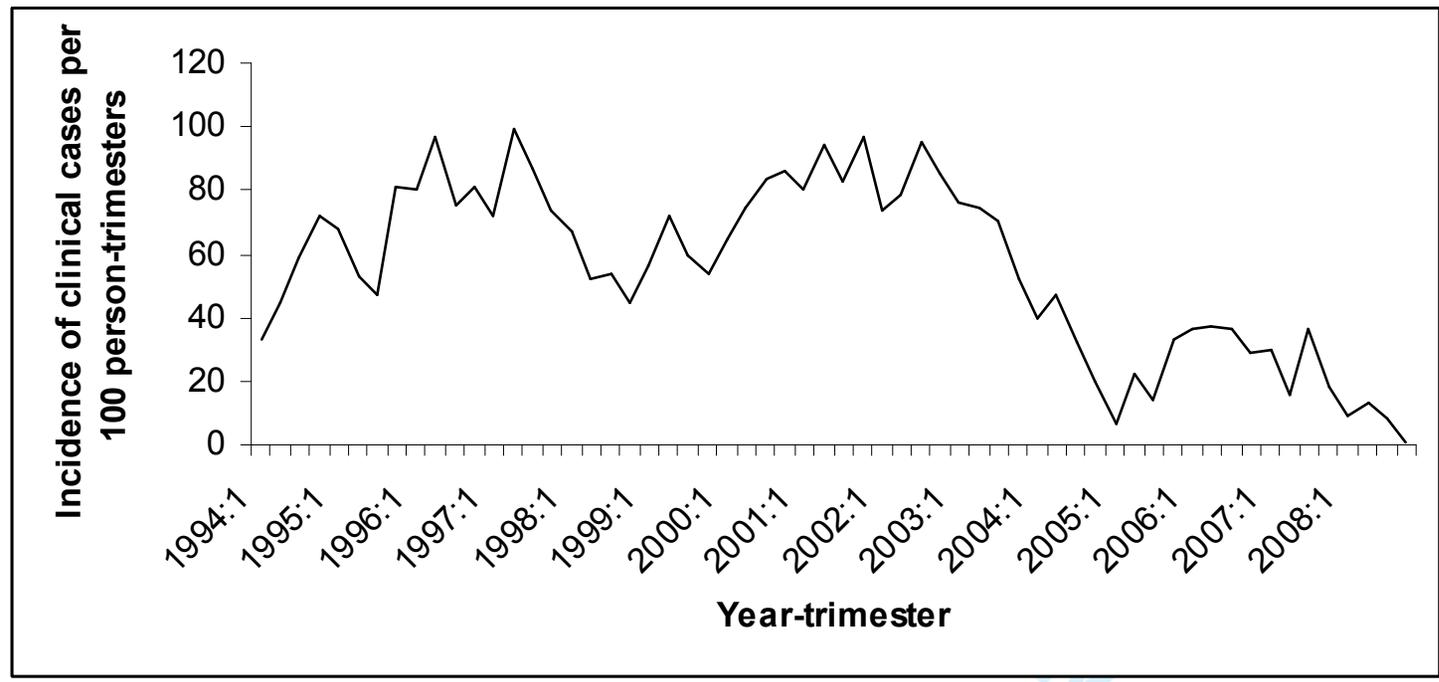
A. Malaria episodes							B. Parasite density				
Age cut-off (years)	OR	95% CI	P value	OR	95% CI	P value	Age cut-off	beta coeff (se)	P value	beta coeff (se)	P value
Atopy				below threshold			above threshold				
1.5	1.80	1.25-2.59	1.7x10 ⁻³	1.57	0.85-2.89	0.15	1.5	0.70 (0.27)	9.2x10 ⁻³	0.54 (0.35)	0.12
2.5	2.00	1.39-2.88	2.0x10 ⁻⁴	1.23	0.76-1.99	0.40	2.5	0.79 (0.26)	2.6x10 ⁻³	0.35 (0.29)	0.23
3.5	2.02	1.39-2.93	2.1x10 ⁻⁴	1.38	0.92-2.08	0.12	3.5	0.85 (0.26)	9.5x10 ⁻⁴	0.37 (0.26)	0.15
4.5	2.10	1.42-3.10	1.6x10 ⁻⁴	1.41	0.98-2.04	0.063	4.5	0.87 (0.25)	6.9x10 ⁻⁴	0.40 (0.23)	0.09
5.5	1.64	1.07-2.52	0.02	1.67	1.17-2.37	0.004	5.5	0.73 (0.27)	7.4x10 ⁻³	0.48 (0.22)	3.4x10 ⁻³
Asthma							Asthma				
1.5	1.98	1.29-3.03	1.8x10 ⁻³	1.46	0.69-3.19	0.34	1.5	0.66 (0.31)	0.03	0.30 (0.44)	0.48
2.5	2.30	1.49-3.55	1.6x10 ⁻⁴	1.15	0.63-2.09	0.65	2.5	0.78 (0.30)	0.01	0.26 (0.36)	0.48
3.5	2.33	1.50-3.61	1.5x10 ⁻⁴	1.50	0.90-2.50	0.12	3.5	0.82 (0.30)	6.2x10 ⁻³	0.43 (0.31)	0.17
4.5	2.30	1.48-3.59	2.4x10 ⁻⁴	1.76	1.11-2.80	0.017	4.5	0.81 (0.29)	5.8x10 ⁻³	0.56 (0.28)	0.049
5.5	1.98	1.22-3.22	0.006	2.06	1.33-3.18	0.0011	5.5	0.72 (0.31)	0.02	0.62 (0.27)	0.02
Atopic Dermatitis							Atopic Dermatitis				
1.5	2.05	1.18-3.56	0.01	0.91	0.42-1.97	0.80	1.5	0.80 (0.37)	0.03	0.72 (0.46)	0.12
2.5	2.49	1.36-4.57	3.1x10 ⁻³	0.82	0.44-1.53	0.53	2.5	0.77 (0.38)	0.044	0.52 (0.39)	0.19
3.5	3.15	1.56-6.33	1.3x10 ⁻³	0.84	0.49-1.46	0.54	3.5	0.99 (0.40)	0.014	0.28 (0.35)	0.42
4.5	3.79	1.61-8.92	2.3x10 ⁻³	0.94	0.57-1.57	0.82	4.5	0.98 (0.47)	0.036	0.29 (0.32)	0.37
5.5	1.33	0.47-3.77	0.59	1.19	0.73-1.96	0.49	5.5	0.26 (0.61)	0.67	0.38 (0.31)	0.22
Rhinoconjunctivitis							Rhinoconjunctivitis				
1.5	1.04	0.66-1.62	0.88	1.01	0.51-2.01	0.98	1.5	0.36 (0.32)	0.27	0.18 (0.41)	0.66
2.5	1.01	0.64-1.61	0.96	0.96	0.55-1.68	0.89	2.5	0.28 (0.33)	0.40	0.25 (0.35)	0.48
3.5	0.95	0.60-1.52	0.83	1.05	0.64-1.72	0.85	3.5	0.31 (0.32)	0.33	0.19 (0.31)	0.54
4.5	0.87	0.54-1.42	0.59	1.06	0.68-1.66	0.79	4.5	0.20 (0.32)	0.53	0.22 (0.28)	0.44
5.5	0.81	0.48-1.36	0.43	1.07	0.70-1.64	0.74	5.5	0.10 (0.33)	0.75	0.23 (0.27)	0.39

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4 **Table S4 Frequency of non-malaria episodes (number of days of presence divided by number of non-malaria episodes) according to allergic status**
5 **and age group.** The *P* value is that from the GLMM analyses of the effect of allergic status by age group on the number of non-malaria episodes per person-
6 trimester.
7

Allergic condition	Allergic status (No/Yes)	Age group (years)		<i>P</i> value
		<3·5	>3·5	
Atopy	N	78·2	85·9	0·105
	Y	87·2	102·6	
Asthma	N	79·6	87·3	0·319
	Y	82·5	100·2	
Atopic dermatitis	N	80·9	88·2	0·323
	Y	73·4	101·9	
Rhinoconjunctivitis	N	77·9	88·3	0·167
	Y	94·9	91·8	

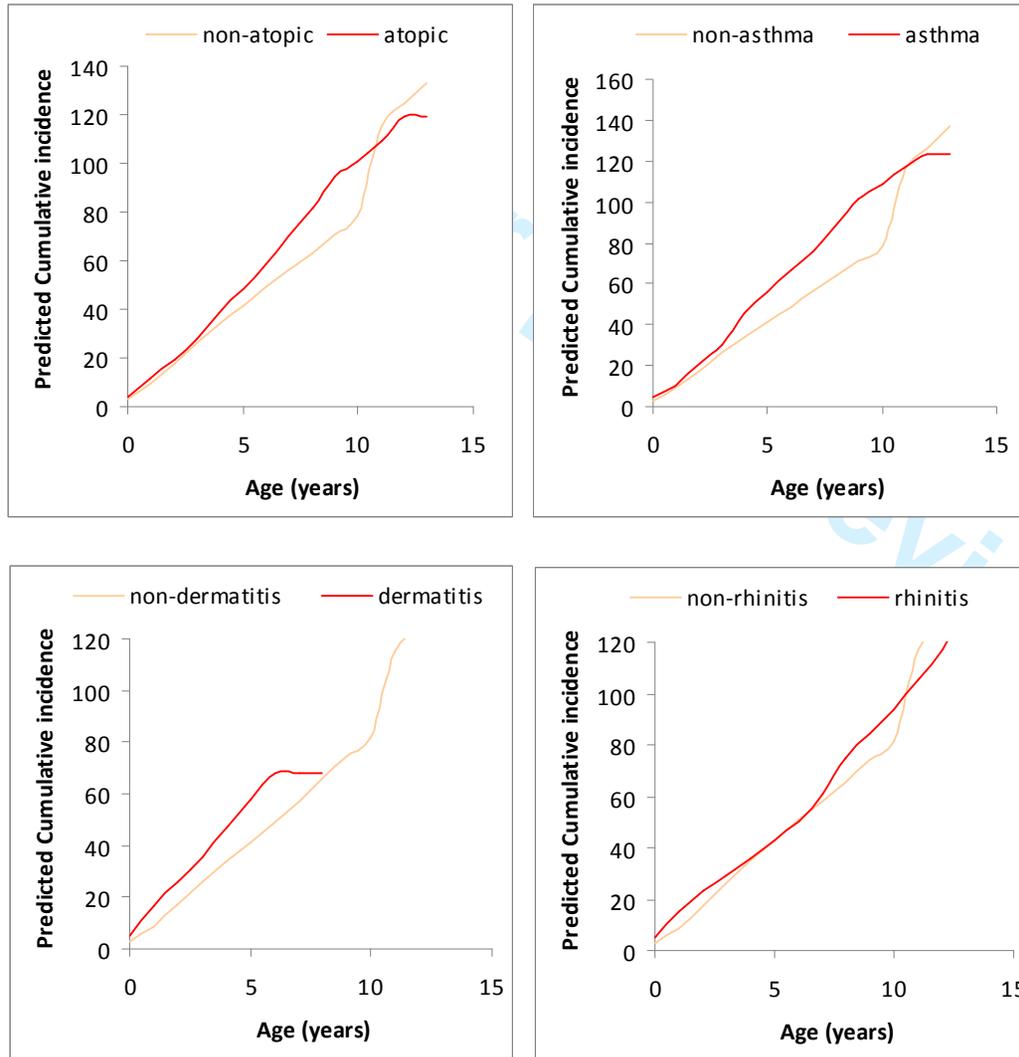
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Figure S1. Incidence of clinical cases per 100 person-trimesters in children under 15 years of age.



Only

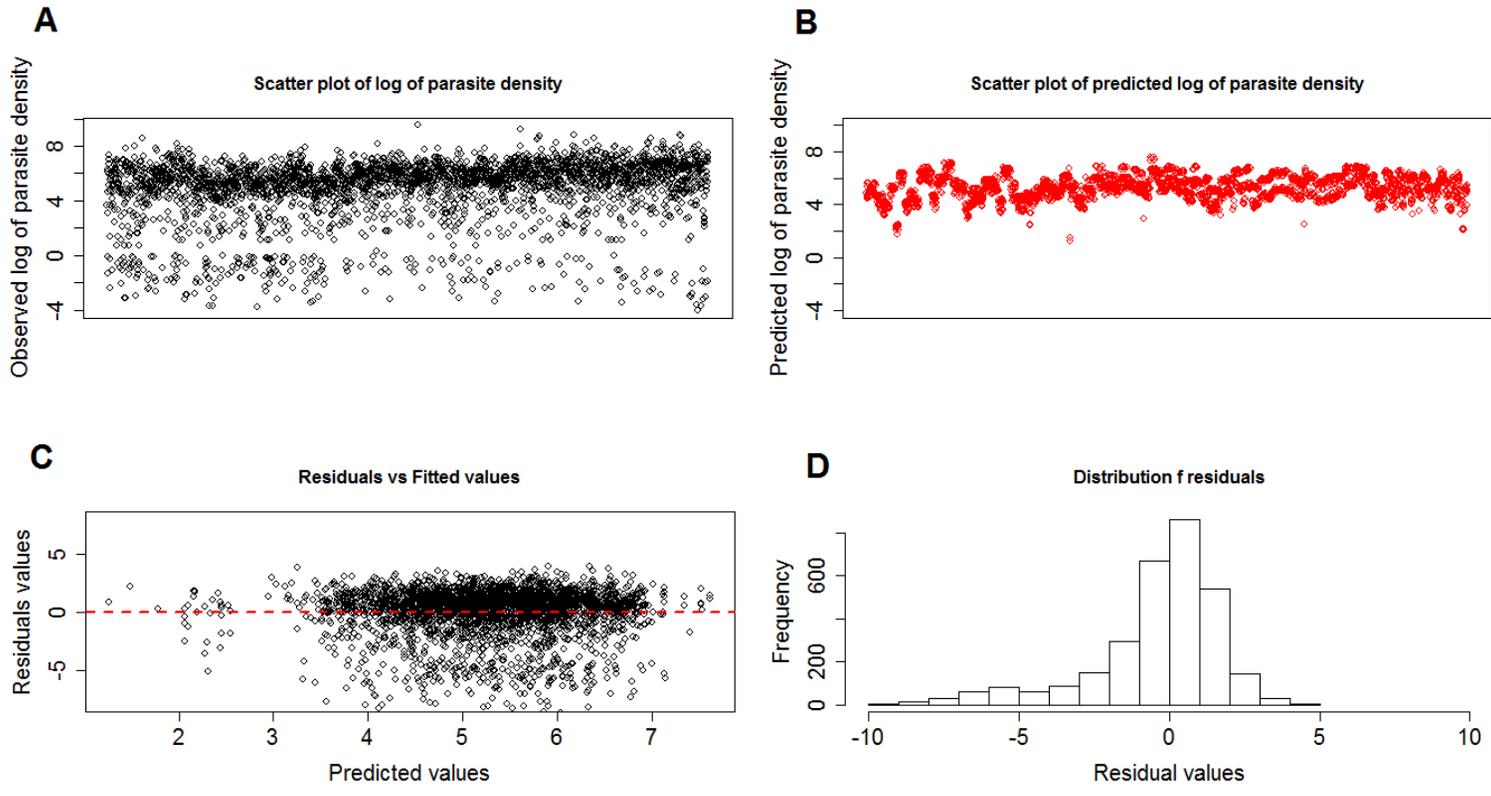
Figure S2. Cumulative incidence of clinical cases according to allergy class predicted by the statistical model.



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Figure S3. Graphical control model for parasite density

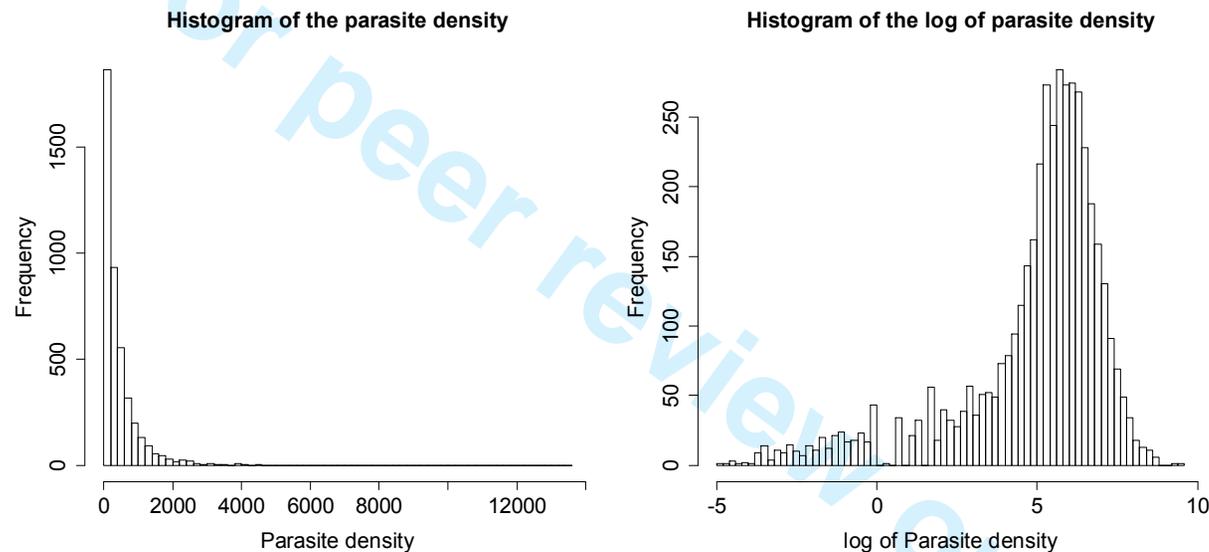
These figures provide a graphical checking of model goodness of fit. Figure A is the scatter plot of the natural logarithm of the observed parasite density and is compared to Figure B, which is the scatter plot of the natural logarithm of the predicted parasite density by the model; on both figures A and B the y-axes give the values for the log of the parasite density. Figure C shows the distribution of the residuals with the predicted values and Figure D is the histogram of the residuals; both figures C and D show the residuals normally distributed around zero.



Analysis using box-cox transformation and probit normalization

The model we fitted on the parasite density ("*pf_density*") has used as outcome variable the natural logarithm of *pf_density* (equivalent to a Box-Cox for which the parameter is null). As shown on Figure S4 the distribution of $\log(pf_density)$ is not perfectly normal, it is left-skewed.

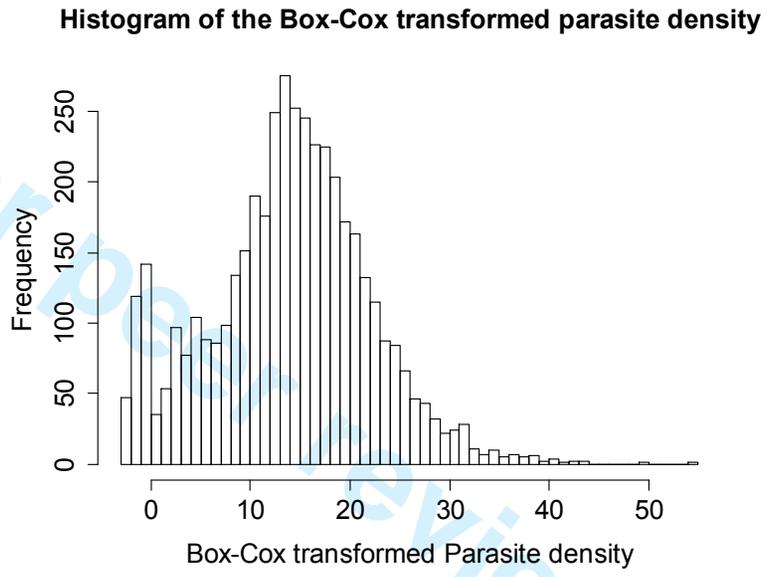
Figure S4. Histogram of *pf_density* and $\log(pf_density)$



We add here the case for a Box-Cox transformation of the parasite density where the parameter is $\lambda = 0.3$, this parameter value was obtained as optimal using the R- function named "boxcox" from the "MASS" library. Then the Box-Cox transformation of the parasite density is $y = (pf_density^{0.3} - 1)/0.3$ having the distribution shown on Figure S5 below.

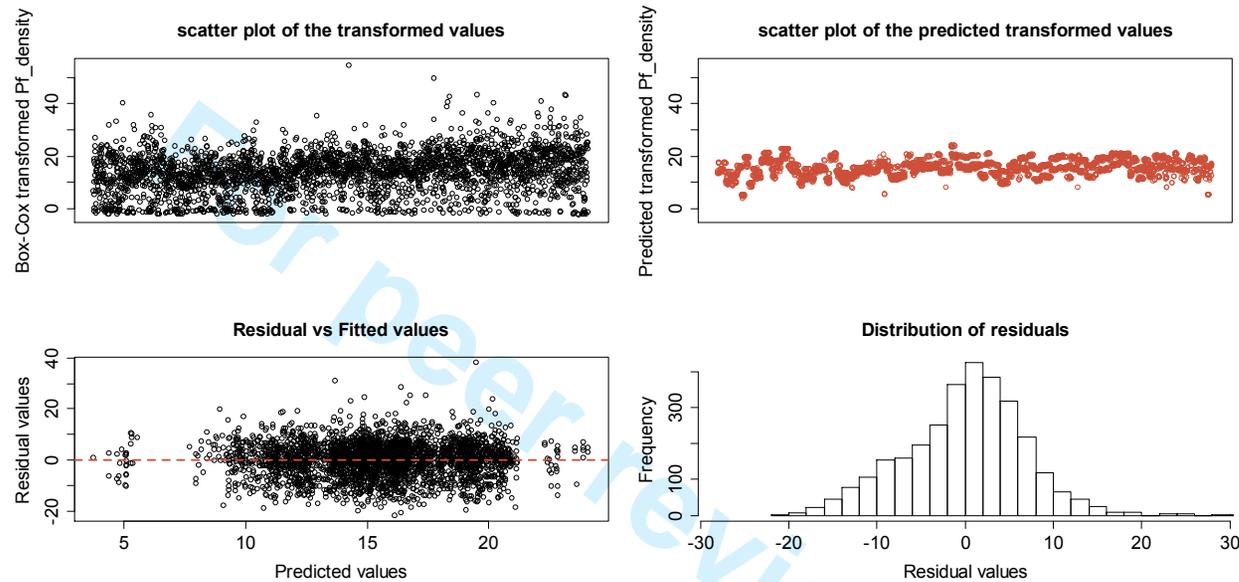
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Figure S5. Histogram of the Box-Cox transformation of pf_density using a λ parameter of 0.3



With this Box-Cox transformed parasitemia as outcome variable, our results are maintained. Note that this distribution is not "perfectly" normal. However, the corresponding graphical control of the model adequation presented on Figure S6 below shows residuals more close to the normal distribution than those for $\log(pf_density)$ as outcome.

Figure S6. Graphical control of the model adequation for $y = \text{Box-Cox}(pf_density, \lambda = 0.3)$

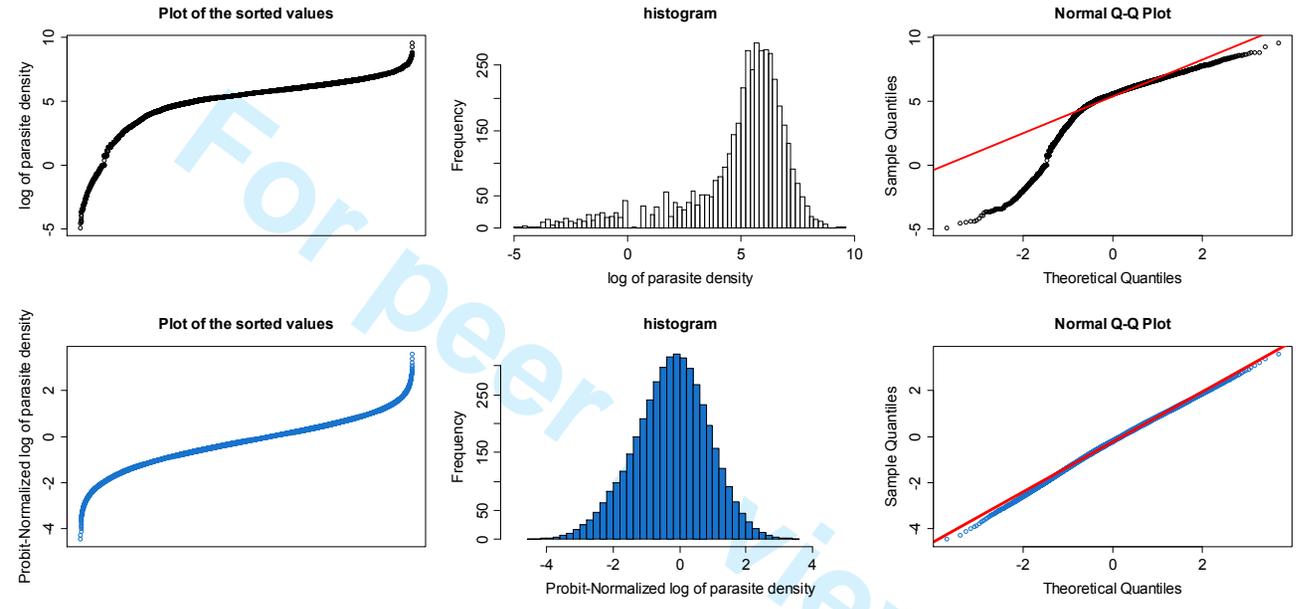


Although using a mixed model approach based on an extreme value distribution would provide a more robust validation of these results, the method we used incorporating pedigree information was developed through an R-package known as "pedigreemm" that allows just for a limited number of distribution laws, which do not include extreme value distributions like the Gumbel or Weibull distributions.

However, we tried the Probit normalization on the $\log(pf_density)$ to readjust its quantiles to those from a standard normal, and subsequently used the derived standard normal transformation of the $\log(pf_density)$ as outcome (see Figure S7 below, the three graphs presented in the first row of the graphs panel concern the $\log(pf_density)$ before Probit normalization and the three in the second row are for after Probit normalization. We can see on the histogram in blue color a good normal distribution of the y variable.

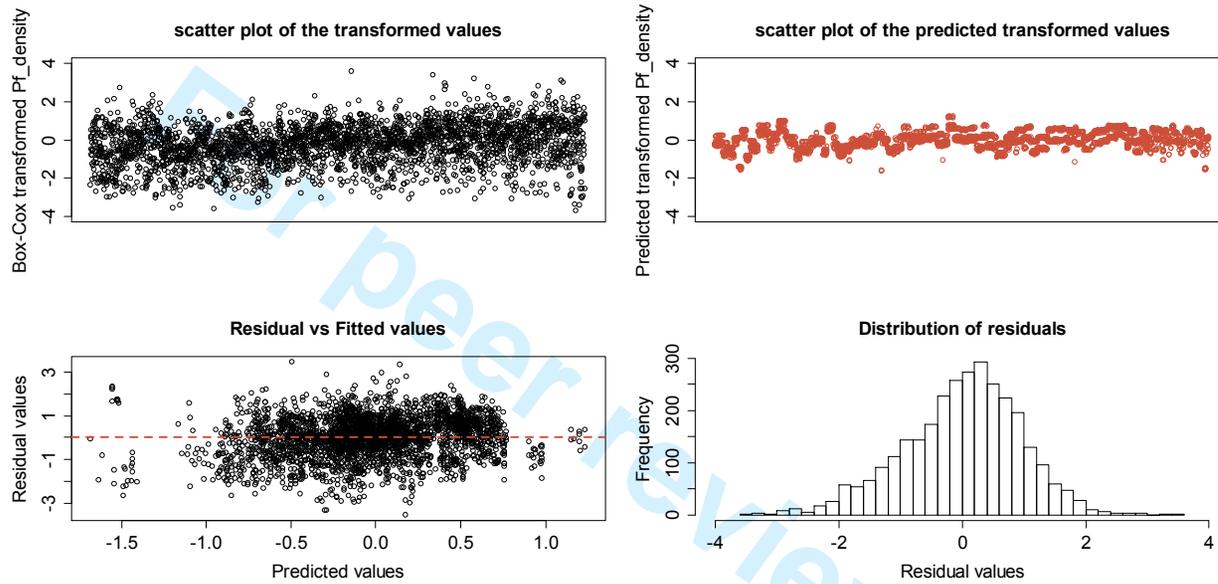
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Figure S7. Probit normalization of the $\log(pf_density)$



The results we obtained after this Probit normalization of the $\log(pf_density)$ confirmed the same findings. Also, the corresponding graphical control of the model adequation presented on Figure S8 below, shows a good normal distribution of residuals from this model.

Figure S8. Graphical control of the model adequation after Probit normalization of the $\log(pf_density)$



Asthma and atopic dermatitis are associated with increased risk of clinical *Plasmodium falciparum* malaria

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Article summary

Article focus

- Genetic studies suggest a link between susceptibility to allergy and malaria in Africa
- We hypothesize that atopy increases susceptibility to malaria

Key messages

- Results demonstrate an association between asthma, atopic dermatitis and susceptibility to clinical *P. falciparum* episodes.
- Genetic pre-disposition to asthma or atopic dermatitis impairs the acquisition of clinical immunity to malaria.
- Administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Strengths and limitations

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association.

Abstract

Objectives: To assess the impact of atopy and allergy on the risk of clinical malaria.

Design: A clinical and immunological allergy cross-sectional survey in a birth cohort of 175 children from 1 month to 14 years of age followed for up to 15 years in a longitudinal open cohort study of malaria in Senegal. Malaria incidence data were available for 143 of these children (aged 4 months to 14 years of age) for up to 15 years. Mixed model regression analysis was used to determine the impact of allergy status on malaria incidence, adjusting for age, gender, sickle cell trait and force of infection.

Main outcome measures: Asthma, allergic rhinoconjunctivitis and atopic dermatitis status, the number of clinical *Plasmodium falciparum* malaria episodes since birth and associated parasite density.

Results: Twelve percent of the children were classified as asthmatic and ten percent as having atopic dermatitis. These groups had respectively a two-fold (OR 2.12 95% confidence intervals 1.46 to 3.08; $P=8 \times 10^{-5}$) and three-fold (OR 3.15, 1.56 to 6.33; $P=1.3 \times 10^{-3}$) increase in the risk of clinical *P. falciparum* malaria once older than the age of peak incidence of clinical malaria (3 to 4 years of age). They also presented with higher *P. falciparum* parasite densities (Asthma: mean 105.3 parasites/ μ L \pm SE 41.0 vs. 51.3 \pm 9.7; $P=6.2 \times 10^{-3}$; Atopic dermatitis: 135.4 \pm 70.7 vs. 52.3 \pm 11.0; $P=0.014$). There was no effect of allergy on the number of non-malaria clinical presentations. Individuals with allergic rhinoconjunctivitis did not have an increased risk of clinical malaria nor any difference in parasite densities.

Conclusion: These results demonstrate that asthma and atopic dermatitis delay the development of clinical immunity to *P. falciparum*. Despite the encouraging decrease in malaria incidence rates in Africa, a significant concern is the extent to which the increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines, administration to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Introduction

The World Allergy Organization estimates that 40% of the world's population is concerned by allergic diseases.¹ In developing countries where *Plasmodium falciparum* malaria is endemic, prevalence of allergy is significantly lower, but is on the increase.² T helper type 2 (Th2) cells, their related cytokines, IgE, eosinophils and mast cells play a major role in allergic inflammation. Orientation of the immune response towards a Th1 profile is crucial for immunity to intracellular pathogens,³ whereas orientation towards a Th2 profile drives immunity to extracellular pathogens and antigens resulting in class switching giving rise to IgE-producing B cells.⁴ ~~An important~~ role of the Th1/Th2 balance in the development of clinical malaria following infection by *P. falciparum* has been suggested by numerous studies.⁵⁻⁷ Whilst it is recognised that acquired anti-parasite immunity is IgG dependent,⁸ it has been suggested that the Th2 bias induced by *P. falciparum* may exacerbate allergy parasite-specific IgE also impact upon the clinical outcome of infection.⁸ For example, higher IgE but not IgG levels have been observed in patients with cerebral malaria than those with uncomplicated *P. falciparum* infection.⁹ The role of IgE, however, remains unclear.¹⁰ Likewise, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*.

~~However,~~ the interplay between infectious agents and allergy is unclear/ambiguous. On the one hand, for example, severe respiratory syncytial virus infection in infants increased the risk of allergic rhinoconjunctivitis and allergic asthma.^{119,120} On the other hand, measles,¹³⁴ hepatitis A¹⁴² and tuberculosis¹⁵³ seemingly reduce atopy. Although, an atopic condition can increase incidence of disease, such as the case for the skin commensal *Staphylococcus aureus* in patients with atopic dermatitis,¹⁶⁴ an atopic tendency *per se* does not generally lead to increased illness from infectious agents.

Genome wide studies have identified chromosomal regions linked to clinical malaria, all of which overlap with those previously identified to be involved in atopic dermatitis, asthma, atopy and IgE levels,¹⁷⁵⁻¹⁹⁷ suggesting that common mechanisms may be involved in both pathologies.²⁰⁴⁸ Chromosomal region 5q31 that has been repeatedly shown to be associated with control of parasite density and contains a cluster of cytokines, among which IL12B has been previously associated with psoriasis.²¹⁴⁹ The other regions, 13q13-q22, 5p15-p13 and 12q21-q23, contain genes involved in innate immunity, notably the interleukin 7 receptor,

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and several involved in tumour necrosis factor synthesis [C1q and tumour necrosis factor related protein 3 (C1QTNF3)] and a gene involved in the complement system (C9).²⁰¹⁸

Several additional lines of evidence support the concept that susceptibility to malaria and atopy may be related to similar immunological defects. In Ethiopia, a history of malaria was associated with atopy.²²⁹ A mouse model for human atopic disease was found to be very susceptible to murine malaria and a major locus for atopic disease mapped close to the region controlling parasite density.²³¹ This region contains several candidate genes that have effects on T-cell function.²³¹

Moreover, a direct effect of histamine in the malaria pathogenesis has been found using genetic and pharmacological approaches²⁴² and increased levels of histamine are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.^{253,264}

To test the hypothesis that allergy impacts upon clinical *P. falciparum* malaria, we performed a clinical allergy cross-sectional study in the family-based longitudinal cohort from Senegal previously used for the genome linkage study²⁰¹⁸ and analysed the impact of asthma, atopic dermatitis, allergic rhinoconjunctivitis on the incidence of clinical *P. falciparum* episodes and the maximum parasite density during each episode.

Methods

Population and outcome data

The malaria research program conducted in Dielmo village in Senegal has been ongoing since 1990 as described elsewhere.²⁷⁵ In brief, between 1990 and 2008, a longitudinal study involving the inhabitants of the village of Dielmo, Senegal, was carried out to identify all episodes of fever. The study design included daily medical surveillance with systematic blood testing of individuals with fever and examination of 200 oil-immersion fields on a thick blood film for malaria parasites (about 0.5 µL of blood). Each individual was given a unique identification code and details of family ties, occupation, and precise place of residence were recorded on detailed maps of each household with the location of each bedroom. All households were visited daily, absenteeism recorded, and the presence of fever or other

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7 symptoms assessed. We systematically recorded body temperature at home three times a
8 week (every second day) in children younger than 5 years, and in older children and adults in
9 cases of suspected fever or fever-related symptoms. In cases of fever or other symptoms,
10 blood testing was done at the dispensary by finger prick, and we provided detailed medical
11 examination and specific treatment. Parasitologically confirmed clinical malaria episodes
12 were treated according to national guidelines. From 1990 to 2008, four different drug
13 regimens were implemented: Quinine from 1990 to 1994, Chloroquine from 1995 to 2003,
14 Fansidar (sulfadoxine-pyrimethamine) from 2004 to mid-2006 and Artemisinin-based
15 combination therapy (ACT; Amodiaquine- sulfadoxine-pyrimethamine) from mid-2006 to
16 2008.

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22 Parasite positivity was established as follows. Thick blood films were prepared and stained
23 by 3% Giemsa stain. Blood films were examined under an oil immersion objective at x1000
24 magnification by the trained laboratory technicians and 200 thick film fields were examined
25 to count the number of asexual and gametocyte parasite stages. Asexual parasite densities
26 (per μL) were calculated by establishing the ratio of parasites to white blood cells and then
27 multiplying the parasite count by 8,000, the average white blood cell count per μL of blood.

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32 Malaria transmission in Dielmo is intense and perennial. We conducted a cross-sectional
33 survey to estimate the prevalence of symptoms related to allergic diseases among 175
34 children aged from 1 month to 14 years old who were born during the malaria research
35 program.

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38 Both the longitudinal and cross-sectional surveys were approved by the Ministry of Health of
39 Senegal. Informed consent of the volunteers is renewed every year. More specifically for the
40 cross-sectional survey, after informing about the procedures and the purpose of the study,
41 written informed consent was obtained from parents or guardians of children either by
42 signature or by thumbprint on a voluntary consent form written in both French and Wolof,
43 the main local language. Consent was obtained in the presence of the school director, an
44 independent witness.

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49 The family structure (pedigree) was available after a demographic census performed for
50 every volunteer at his adhesion in the project. A verbal interview of mothers or key
51 representatives of the household was used to obtain information on genetic relationships
52 between studied individuals, their children, their parents, and to identify genetic links
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among the population. The total pedigree comprised 828 individuals, including absent or dead relatives, composed of ten independent families that can be sub-divided into 206 nuclear families (father – mother couples with at least one child) with an average of 3.6 children each. Genetically related nuclear families occur because of multiple marriages and marriages among related individuals. Previous typing with microsatellites has enabled the construction of a pedigree based on Identity-by-Descent using MERLIN.^{2048,286} The mean coefficient of inbreeding is 0.0008. Newborns since this original genetic analysis were added to the family of the parents in question. The 143 children, with both allergy and malaria data, belonged to 61 nuclear families and comprised 30 singletons, 102 siblings and 11 half-sibs (yielding 55 half-sib pairs). The mean genetic relatedness (by pedigree) of the 143 children is 0.0114 (range: 0.0013 to 0.022).

P. falciparum clinical episodes

P. falciparum malaria clinical episode phenotypes analysed were: (i) clinical *P. falciparum* infections treated with anti-malarial therapy and (ii) the highest parasite density during the *P. falciparum* clinical episode. A clinical *P. falciparum* episode was defined as a clinical presentation with fever (axillary temperature $\geq 37.5^{\circ}\text{C}$) and/or other clinical signs suggestive of malaria associated with a thick blood smear positive for *P. falciparum* and that was treated with anti-malarial therapy. Repeated clinical malaria presentations within 15 consecutive days were not considered to be independent and were excluded from the analyses, unless there was a negative thick blood smear between two clinical presentations. We also excluded observations in any trimester for which the individual was not present for at least one third of the time.

We calculated the quarterly incidence rate of clinical *P. falciparum* episodes in children below the age of 15 years as the ratio of the total number of clinical *P. falciparum* episodes during the trimester divided by the total number of person-trimesters surveyed. Incidence rate is expressed as cases per 100 person-trimesters (see Supplementary Figure S1). This rate was used in the analysis to approximate the force of infection (exposure level) within the targeted population at the time of a given clinical *P. falciparum* episode.

The total number of clinical presentations per trimester that were not attributable to *P. falciparum* was tabulated. Repeated non-malaria presentations within seven consecutive days were not considered to be independent and were excluded.

Allergic diseases and atopic status

The International Study of Asthma and Allergies in Childhood (ISAAC) diagnostic criteria have been shown to be reproducible, adequate and able to discriminate children with allergic diseases in different areas of the world.² The standardized ISAAC questionnaire originally written in English was translated into French in compliance with ISAAC guidelines²⁹⁷, adapting it to the usual local customs following advice from local clinicians and paediatric allergologists (Acknowledgements and Technical Appendix). The adequacy and reliability of the translated questionnaire had been previously confirmed by a pilot study on 30 randomly selected children in the same community. The questionnaire was completed by specially trained health workers during an oral interview conducted in Wolof with children and their mothers or guardians.

To assess the prevalence of allergic diseases in children, we used the positive and negative predictive values of the ISAAC questionnaire diagnosis criteria developed for subtropical countries.³⁰²⁸ Each question was scored according to the medical diagnosis of paediatricians and paediatric allergologists. Positive or negative answers were thus graded on the basis of symptom sensitivity, specificity, frequency, location or early onset. For each allergic disease, three categories of symptom severity, *severe*, *moderate*, and *none*, were defined as follows:

Asthma – *severe* symptoms if the child had “wheezing or whistling in the chest before the age of two years” and “more than three times” or severe enough to “limit his/her speech”; *moderate* symptoms if the child had “wheezing or whistling in the chest before the age of two years” and “in the past 12 months”; and *none* otherwise.

Allergic rhinoconjunctivitis – *severe* symptoms if the child had “sneezing, runny or stuffy nose in the past 12 months” and “more than five times a year”, and “itchy, watery eyes or tropical endemic limboconjunctivitis (TELC) in the past 12 months”; *moderate* symptoms if the child had “sneezing, runny or stuffy nose in the past 12 months”, and “itchy, watery eyes or TELC in the past 12 months”; and *none* otherwise.

Atopic dermatitis – *severe* symptoms if the child had “scaly or exudating, crusted and pruritic patches in the past 12 months” and “affecting any of the following characteristic areas: face, around the ears or eyes, folds of armpits or elbows or groin, behind the knees, under the buttocks”, and “onset of symptoms before the age of two years”; *moderate* symptoms if the

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7 child had “scaly or exudating, crusted and pruritic patches in the past 12 months” and
8 “affecting any of characteristic areas (see above)”, and “onset of symptoms before the age
9 of four years”; and *none* otherwise.

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11 The inter-relationships between variables reflecting the severity of symptoms of the three
12 allergic diseases were used to identify children at high risk of atopy. The *high probability*
13 group was defined by the prevalence of at least one of any *severe* symptoms or two of any
14 *moderate* symptoms. The *probable* group was defined as those with *moderate* symptoms
15 from one of the three allergic diseases and remaining children were classified in the *unlikely*
16 group.

21 *Helminths*

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23 Helminthic infections are common in this region and are known to modify the clinical course
24 and outcome of both allergic diseases and malaria.^{31,29,329} We therefore carried out a
25 helminth survey for 91 individuals present during the cross-sectional survey. Diagnosis was
26 performed by stool examination by microscope and by the Kato technique to search for the
27 presence of *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator*
28 *americanus*), whipworm (*Trichuris trichiuria*), *Schistosoma mansoni*, and *Strongyloides*
29 *stercoralis*. Examination for pinworms (*Enterobius vermicularis*) was performed by the anal
30 scotch-test. An anti-helminthic treatment was proposed for all infested individuals.

36 *Immunoglobulin E titres*

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38 Specific IgE titres were measured by ELISA as previously described.³³¹ A panel of allergens of
39 potential pertinence to the three classes of allergy was used: (i) Salivary gland extracts (SGE)
40 of two mosquito species present in the study cohorts, *Aedes aegypti* and *Anopheles gambiae*
41 *sensu stricto*, and (ii) *P. falciparum* parasite extract were prepared as previously described³¹;
42 (iii) House dust mite spp. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*;
43 (iv) a mix of pollen allergens from five ubiquitous gramineae spp. [Cock's-foot (*Dactylis*
44 *glomerata*), Timothy grass (*Phleum pratense*), Sweet Vernal grass (*Anthoxanthum*
45 *odoratum*), Perennial ryegrass (*Lolium perenne*), Kentucky Bluegrass (*Poa pratensis*)] (all
46 from Stallergenes, France).

52 **Statistical analysis**

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7 Statistical analyses were performed using R version 2.12.0 (The R Foundation for Statistical
8 Computing, Vienna, Austria). To address the effect of allergic status on the risk of clinical *P.*
9 *falciparum* episodes, we performed Generalized Linear Mixed Models (GLMM) extended to
10 pedigree data using the *pedigreemm* package for R to account for the non-independence of
11 individuals because of family relationships, shared house and for repeated measures from
12 the same individual (Technical Appendix). Correlated individual effects due to familial
13 relationships were taken into account by using the pedigree-based genetic relatedness
14 matrix that contains the genetic covariance among all pairs of individuals in the study cohort
15 and is calculated using the pedigree information.³⁴² Shared house and repeated measures
16 from the same individual were modelled as random effects. All random effects were
17 assumed to be normally distributed, and conditional on these random effects, the
18 dependent variable had: (i) a Binomial distribution when the studied phenotype was the
19 occurrence of a clinical *P. falciparum* episode treated with anti-malarial therapy during a
20 trimester, (ii) a Gaussian distribution when the studied phenotype was the logarithm of the
21 maximum parasite density during a given clinical *P. falciparum* episode, and (iii) a Poisson
22 distribution when the studied phenotype was the number of non-malaria episodes per
23 trimester. The effects of allergy disease classes on these dependent variables were modelled
24 as fixed effects. Allergy classes were reduced to two levels, *Severe* or *moderate vs. none* for
25 analyses of asthma, atopic dermatitis and allergic rhinoconjunctivitis and *high probability vs.*
26 *probable* and *unlikely* for atopic tendency. Co-variables included sickle cell trait³³¹, gender,
27 number of days present on site during the trimester, trimestrial incidence of *P. falciparum*
28 and age. Age was initially analysed as a continuous covariate. To assess the age-specific
29 effect of allergy, age was categorised into two levels (<3.5 years of age and ≥3.5 years of
30 age, based on the age of peak clinical incidence) and allergy class was nested within age
31 class. The age threshold was varied from 1.5 years to 5.5 years of age and the data re-
32 analysed to assess at which age there was the strongest effect. The association of allergy
33 classes with IgE levels was analysed by box-cox transforming the data and fitting a GLMM
34 with a normal distribution.
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52 Results

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Of the 205 eligible children aged under 15 years involved in the family-based longitudinal study, 175 (85.4 %) participated in the cross-sectional survey to assess the prevalence of related symptoms of allergic diseases. All eligible children present at the time of the survey were included; no explicit refusal to participate was recorded. The study cohort was aged from 1 month to 14 years 11 months. The sex-ratio (male/female) was 0.94.

From 1994 until 2008, 143 of the children participating in the cross-sectional survey were present for at least 31 days in any trimester during the study period generating a total of 3,093 person-trimesters of presence (Supplementary Table S1). There were 2,065 treated *P. falciparum* clinical episodes (per individual: median 11, range 0-47)(Supplementary Table S2). The age peak of incidence of *P. falciparum* episodes occurred at 3 to 4 years of age (Figure 1). There were 1,868 non-malaria episodes (median 12, range 0-37) (Table S2). These non-malaria clinical presentations were associated with headache (38 %), chills (32 %), cough (13 %), vomiting (11 %) and diarrhoea (6 %).

The prevalence of moderate or severe asthma symptoms was respectively 2.3 % and 10.3 % (Table 1). The prevalence of moderate or severe allergic rhinoconjunctivitis symptoms was respectively 6.3 % and 10.3 %. The prevalence of moderate or severe atopic dermatitis symptoms was respectively 6.3 % and 2.9 %. On the basis of symptom severity, an atopic tendency was estimated to be unlikely for 68.0 %, probable for 9.1 % and highly probable for 22.9 % of the 175 children. The frequency of each allergy class in children for whom malaria data were available is shown in Table S1.

The risk of treated clinical *P. falciparum* infections was higher for children with high probability of atopy (OR 1.65, 95% confidence intervals 1.20 to 2.26; P=0.002) (Table 2), after adjusting for age, sickle cell trait and the exposure level. Gender was not found to be significant. Analysing the impact of atopy in children younger and older than the peak age of clinical incidence (3 to 4 years old), revealed that atopy increased the risk of *P. falciparum* episodes in children at an age greater than 3.5 years (OR 2.02, 1.39 to 2.93; P=2x10⁻⁴), but not in children of age prior to the peak clinical incidence (OR 1.38, 0.92 to 2.08; P=0.124) (Table 2). This increased risk resulted in an ever increasing cumulative number of *P. falciparum* episodes with age beyond that of peak clinical incidence (Figure 2. See supplementary Figure S2 for model predictions for comparison).

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7 Analysis by allergy category revealed that asthma (severe or moderate) increases the risk of
8 *P. falciparum* episodes (OR 2.12, 1.46 to 3.08; $P= 8 \times 10^{-5}$) and this again only in children of
9 age greater than 3.5 years old (OR 2.33, 1.50 to 3.61; $P= 1.5 \times 10^{-4}$). Atopic dermatitis
10 increased the risk of clinical malaria in children older (OR 3.15, 1.56 to 6.33; $P= 1.3 \times 10^{-3}$) but
11 not younger than 3.5 years of age (Table 2). Allergic rhinoconjunctivitis was not associated
12 with increased risk of clinical malaria at any age (Table 2). The impact of atopy, asthma and
13 atopic dermatitis can be clearly seen in the ever-increasing number of cumulative *P.*
14 *falciparum* episodes beyond the age of the onset of clinical immunity in the population, 3.5
15 years of age (Figure 2). There is no difference in the number of clinical malaria episodes prior
16 to this age in individuals with or without an allergic condition. Analysis using different age
17 thresholds (from 1.5 to 5.5 years of age) revealed similar OR for thresholds of 2.5, 3.5 and
18 4.5 years of age. The maximum OR for increased malaria occurred in children older than 4.5
19 years of age and with atopy or atopic dermatitis, whereas for the asthma group it occurred
20 in children after 3.5 years of age (Supplementary Table S3).

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28 There was no impact of any allergic disease on the number of non-malaria episodes by
29 trimester (Supplementary Table S4).

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32 The impact of atopy, asthma and atopic dermatitis on the maximum *P. falciparum* parasite
33 density during a given clinical malaria episode mirrored that of the risk of *P. falciparum*
34 episodes. Parasite density was significantly higher for children with allergic disease older
35 than 3.5 years of age (Table 3 and supplementary Figure S3 for residuals of the fitted model).

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38 As the log-transformed data were left skewed, we additionally analysed using box-cox
39 transformation and probit normalization of the data. The results were qualitatively the same
40 (Supplementary text and Figures S4-S8). Allergic rhinoconjunctivitis had no impact on the
41 parasite density (Table 3). Analysis using different age thresholds yielded ~~the same~~
42 patterns similar qualitative conclusions as seen with the number of clinical episodes (Table
43 S3).

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48 Individuals with moderate or severe symptoms of atopic dermatitis had significantly higher
49 specific IgE titres against *Ae. aegypti* ($P=0.004$) and *An. gambiae* SGE ($P<0.001$). There were
50 no detectable specific anti-*P. falciparum* IgE. Individuals with moderate or severe symptoms
51 of allergic rhinoconjunctivitis did not have significantly higher IgE titres against the tested
52 gramineae ($P=0.28$), although titres decreased with age ($P=0.035$). There was also no effect of
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asthma on IgE titres against the house dust mite spp. tested (*D. farinae* P=0.60 & *D. pteronyssinus* P=0.27).

Only five individuals were infested with helminths (two *Ancylostoma*, one *Strongyloides*, one *Trichuris* and one *Enterobius*).

Discussion

Principal findings

Establishing the allergic status of children up to the age of 15 years old followed for malaria since birth, revealed an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

Strengths and weaknesses of the study

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association. In addition, although allergy diagnosis for children under 2 years of age is not considered reliable, there were only 15 individuals under 2 at the time of the allergy study of the 143 for whom malaria and allergy data were available.

Meaning of the study

Under intense malaria transmission, after repeated exposure to the parasite, children develop a clinical immunity³⁵³, whereby they tolerate elevated parasite densities without showing clinical symptoms. In this cohort, the population mean onset of clinical immunity occurred at 3 to 4 years of age. Although clinical immunity is accompanied by a reduction in parasite density, effective anti-parasite immunity develops much more slowly³⁶⁴ with individuals achieving a state of premunition, whereby they maintain low-grade parasite densities in an asymptomatic state.³⁷⁵ We show here that children with clinically defined

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7 | asthma or atopic dermatitis ~~had a two to three fold~~have an increased ~~in the~~ risk of
8 presenting with *P. falciparum* malaria episodes requiring treatment once passing the age of
9 peak clinical incidence. They also had higher parasite density during clinical episodes,
10 suggesting a reduced ability to control parasite replication. The observed increase in clinical
11 incidence of malaria in patients with asthma or atopic dermatitis is not likely to be the result
12 of increased frailty of such individuals; these individuals did not come more frequently to the
13 clinic with non-malaria symptoms. Our previous genome linkage study identifying
14 chromosomal regions²⁰⁴⁸ associated with malaria that overlap with those previously shown
15 to be linked to asthma/atopy suggests that there may be a shared genetic basis to these
16 pathologies rather than any causative effect of one on the other. This is consistent with the
17 increased susceptibility to malaria of mouse atopic models.²³⁴

23 24 **Comparison with other studies**

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26 | A previous study in Ethiopia (East Africa) found that a history of malaria (yes/no) increased
27 risk of atopic dermatitis in 306 cases compared to 426 controls as characterized using the
28 ISAAC questionnaire.²²⁹ The only other epidemiological study that has previously examined
29 the link between malaria and atopy³⁸⁶ also interpreted the result from the perspective of the
30 impact of malaria on atopy. They examined the re-infection rate with *P. falciparum* over a 5-
31 year period in 91 children that were subsequently classified as atopic or not using skin prick
32 tests (SPT) with house dust mite antigen. Their conclusion was that, as with measles¹³⁴ and
33 tuberculosis¹⁵³, malaria infection reduces atopy. However, the study lacked previous
34 infection data since birth of the participating individuals and focussed on atopy as
35 determined by SPT against a single allergen. The case-control study of atopic dermatitis risk
36 factors cited above found no overall association between allergen skin sensitization and
37 atopic dermatitis. We also found no evidence of increased IgE titres against house dust mites
38 in the asthmatic or atopic dermatitis groups or against grass pollen in individuals with
39 allergic rhinoconjunctivitis. Such differences likely reflect the different IgE reactivity profiles
40 due to differences in allergen exposure in Africa.³⁹⁷ There was no evidence of anti-parasite
41 IgE in this cohort of children. We previously showed that circulating anti-parasite IgE titres
42 were strongly positively correlated with anti-mosquito saliva IgE, but became undetectable
43 following malaria exposure, potentially being bound to effector cells.³³⁴ Only mosquito
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7 saliva, a known major local allergen, induced a specific IgE response at significantly higher
8 titres in individuals with atopic dermatitis.
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10 Although the immune effectors of clinical immunity are still poorly defined, there is strong
11 evidence that acquired anti-parasite immunity is IgG-dependent³⁸ and cytophilic
12 immunoglobulins (IgG1 & IgG3), which are capable of eliminating the parasites by
13 opsonisation and/or by Antibody Dependent Cellular Immunity play an important role in
14 premunition.³⁷⁵ The higher parasite density during symptomatic episodes observed in the
15 asthma group suggests impaired development of acquired immunity. Impaired acquisition of
16 immunity to malaria in children with asthma or atopic dermatitis may stem from their
17 imbalanced Th1/Th2 response. Indeed, an atopic state may generate a tendency to develop
18 a Th2 type immune response to *P. falciparum*. Dendritic cells that are oriented to a Th2
19 phenotype are more susceptible to orient the acquired immune response towards a Th2
20 profile.⁴⁰³⁹ Orientation of the immune response towards a Th2 profile by asthma or atopic
21 dermatitis would result in a poor Th1 response (and hence development of protective IgG
22 immunoglobulins), considered to be the dominant arm of the immune response enabling
23 resistance to infectious disease in children.⁴¹⁹
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32 Many studies have revealed an important role of histamine, a key downstream effector
33 molecule in allergic reaction, in the outcome of a malaria parasite infection.^{242-264,421-454}
34 Moreover, reports indicate that components of the innate immune system, including
35 eosinophils, basophils, and mast cells (MCs), could play important roles in the pathogenesis
36 of malaria.⁴²¹ Increased levels of histamine in plasma and tissue, derived from basophils and
37 MCs, notably following stimulation by IgE through the high affinity receptor FcεR1, are
38 associated with the severity of disease in humans infected with *P. falciparum* and in animal
39 malaria models.^{253,264} Chlorpheniramine, a HR1agonist reversed resistance to chloroquine
40 and amodiaquine both *in vivo* and *in vitro*.⁴³² Moreover, astemizole, another HR1 agonist,
41 was identified as an anti-malarial agent in a clinical drug library screen.⁴⁴³ Finally, *P.*
42 *falciparum* produces translationally controlled tumor protein, which is a homolog of the
43 mammalian histamine-releasing factor that causes histamine release from human
44 basophils.⁴⁵⁴
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52 Further research

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7 Our results provide the first birth cohort study addressing the link between malaria and
8 allergic diseases. They contribute to a growing body of evidence that the pathologies are
9 related. ISAAC has revealed a steady but significant increase in prevalence rates of asthma
10 and allergic diseases in Africa. Whilst the majority of studies have focused on large cities,
11 there is increasing urbanization throughout Africa, as well as improved access to primary
12 health care in many areas. A key concern for ISAAC is the extent to which such societal
13 evolution will result in an increase in allergic diseases. Increased urbanization in sub-Saharan
14 Africa is changing the epidemiology of malaria and although resulting in a decrease in risk,
15 will result in more severe clinical malaria in older individuals.^{465,476} Moreover, a large
16 consumption of anti-malarial drugs in the urban areas provides substantial drug pressure
17 fostering, the selection of drug-resistant parasites. Despite the encouraging recent decrease
18 in malaria incidence rates, even in rural areas, an additional significant concern is the extent
19 to which such an increase in allergy will exacerbate the burden of malaria. Given the
20 demonstrated anti-parasitic effect of anti-histamines^{47,48}, administration of anti-histamines
21 to atopic children will likely reduce the burden of clinical malaria in these children, increase
22 the efficacy of first-line treatment anti-malarials⁴⁹⁸ and alleviate the non-infectious
23 consequences of atopy. Clinical intervention studies should be envisaged.

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33 What is already known on this topic

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35 There are several reports of the beneficial effects of anti-histamines for malaria
36 chemoprophylaxis^{242-264,487} as well as our previous work²⁰¹⁸ showing that chromosomal
37 regions associated with malaria are also linked to allergy and atopy.¹⁷⁵⁻¹⁹⁷ There are two
38 epidemiological studies showing opposite effects of malaria on atopy.^{229,386}

41 What this study adds

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43 Using a longitudinal malaria study birth cohort, we identified an association of asthma and
44 atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the
45 increase in risk of malaria associated with these allergic conditions occurred only after the
46 peak clinical incidence of disease in the population, suggesting that they delay the
47 development of clinical immunity to malaria.

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Contributors: LB, SM, and RP made substantial contributions to the concept and design of the study. MH, HB, BG, SB, FDS, and AT were involved in acquisition of the data. CL, AF, OMP, AS and RP contributed to the analysis and interpretation of the data. MH, CL, HB, BG, SB, FDS, AF, AT, LB, OMP, SM, AS and RP critically reviewed the report and approved its final version for submission. All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. MH and RP are guarantors.

Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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7 Ethical approval: The allergy study was approved by the Senegalese National Ethics
8 committee (2009/N°46). Renewed approval of the longitudinal malaria study was obtained
9 from the same committee (2006/N°969).
10

11 Data sharing: The allergy database will be made available on-line. The longitudinal malaria
12 data set will be made available following discussion with the coordinators of the three
13 Institutes that govern the dataset through contact with the corresponding author.
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Table 1 Classification of Asthma, Allergic rhinoconjunctivitis, Atopic dermatitis and overall Atopic status according to ISAAC questionnaire in children aged 0-14 from a malaria birth cohort. N is total number of children examined and n-malaria represents those for whom malaria data were recorded. F is the number of females and M the number of males.

	N (F/M)	%	n-malaria (F/M)
Asthma symptoms			
None	153 (73/80)	87.43	125 (59/66)
Moderate	4 (1/3)	2.29	4 (1/3)
Severe	18 (6/12)	10.29	14 (4/10)
Rhinoconjunctivitis symptoms			
None	146 (64/82)	83.43	120 (52/68)
Moderate	11 (8/3)	6.29	9 (6/3)
Severe	18 (6/12)	10.29	14 (6/8)
Atopic dermatitis symptoms			
None	159 (75/84)	90.86	128 (60/68)
Moderate	11 (1/10)	6.29	11 (1/10)
Severe	5 (4/1)	2.86	4 (3/1)
Atopic tendency			
Unlikely	119 (56/63)	68.00	97 (46/51)
Probable	16 (8/8)	9.14	14 (6/8)
Highly probable	40 (16/24)	22.86	32 (12/20)

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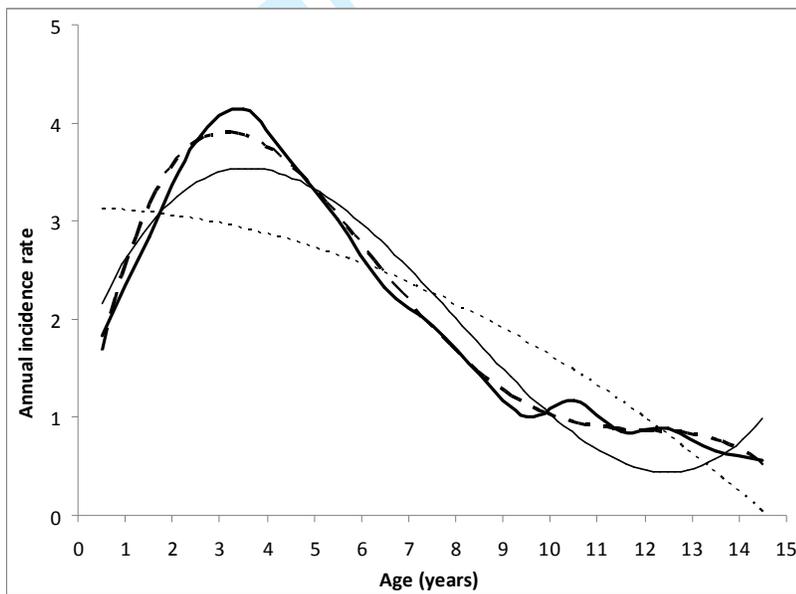
Table 2 Impact of allergy status on risk of *P. falciparum* clinical episodes. Shown are the *P* values and adjusted Odds Ratios with 95% confidence intervals calculated from the mixed model analyses. Values for the covariables Age (≥ 3.5 years of age compared to < 3.5 years of age), Trimestrial incidence of *P. falciparum* clinical episodes and HbAS (beta-globin sickle cell trait; AS compared to AA) are those from the Asthma model analysis. For clarity significant co-variables are shown in bold.

	Age groups < 3.5 years $>$	ORa	95% Confidence Intervals		<i>P</i> value
			Lower	Upper	
Atopy	Both	1.65	1.20	2.26	2.0×10^{-3}
	< 3.5	1.38	0.92	2.08	0.124
	≥ 3.5	2.02	1.39	2.93	2.1×10^{-4}
Asthma	Both	2.12	1.46	3.08	8.0×10^{-5}
	< 3.5	1.50	0.90	2.50	0.122
	≥ 3.5	2.33	1.50	3.61	1.5×10^{-4}
Atopic dermatitis	Both	1.05	0.65	1.70	0.842
	< 3.5	0.84	0.49	1.46	0.539
	≥ 3.5	3.15	1.56	6.33	1.3×10^{-3}
Rhinoconjunctivitis	Both	0.96	0.65	1.41	0.818
	< 3.5	1.05	0.64	1.72	0.853
	≥ 3.5	0.95	0.60	1.52	0.834
Age ≥ 3.5		0.48	0.40	0.57	2.7×10^{-15}
Trimestrial incidence		1.01	1.00	1.01	1.8×10^{-6}
HbAS		0.24	0.12	0.47	3.7×10^{-5}

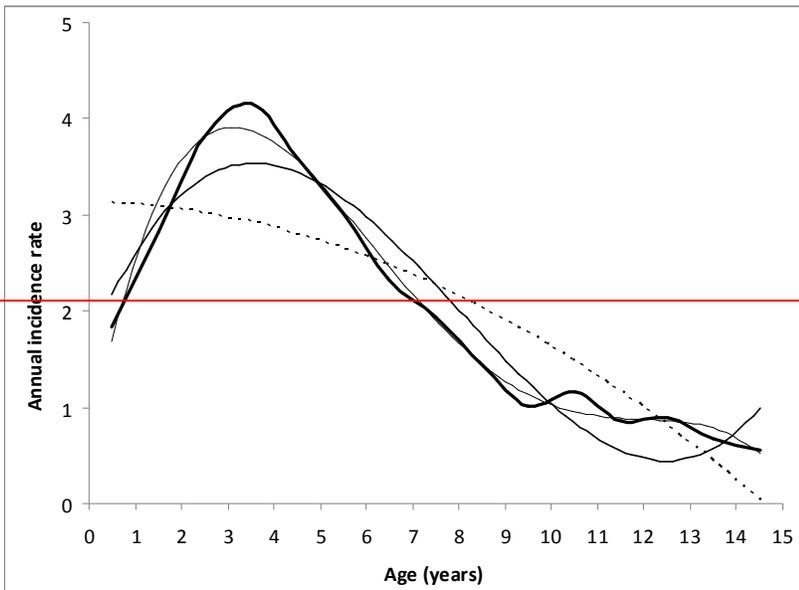
Table 3 Impact of allergy status on the maximum *P. falciparum* parasite density during a clinical malaria episode. Shown are the back-transformed mean parasite densities per microlitre and standard errors (SEM) estimated from the GLMM analyses after taking into account the other co-variables. Significantly different effects are shown in bold for clarity.

Allergic condition	Age groups	Allergic status (No/Yes)	Mean parasite density	SEM	P value
Atopy	Both	N	76.3	13.8	
		Y	131.0	36.4	0.0158
	<3.5	N	114.3	23.7	
		Y	171.1	56.0	0.148
	≥3.5	N	48.4	9.8	
		Y	114.8	37.1	9.5x10⁻⁴
Asthma	Both	N	78.1	14.4	
		Y	148.5	44.3	3.8 x10⁻³
	<3.5	N	114.8	24.3	
		Y	171.9	74.5	0.167
	≥3.5	N	51.3	9.7	
		Y	105.3	41.0	6.2 x10⁻³
Atopic dermatitis	Both	N	82.6	15.0	
		Y	93.9	38.9	0.605
	<3.5	N	122.6	25.5	
		Y	133.9	63.5	0.425
	≥3.5	N	52.3	11.0	
		Y	135.4	70.7	0.014
Rhinconjunctivitis	Both	N	81.5	14.8	
		Y	111.4	39.0	0.570
	<3.5	N	118.8	25.1	
		Y	166.3	69.9	0.537
	≥3.5	N	54.6	11.3	
		Y	80.9	33.7	0.327

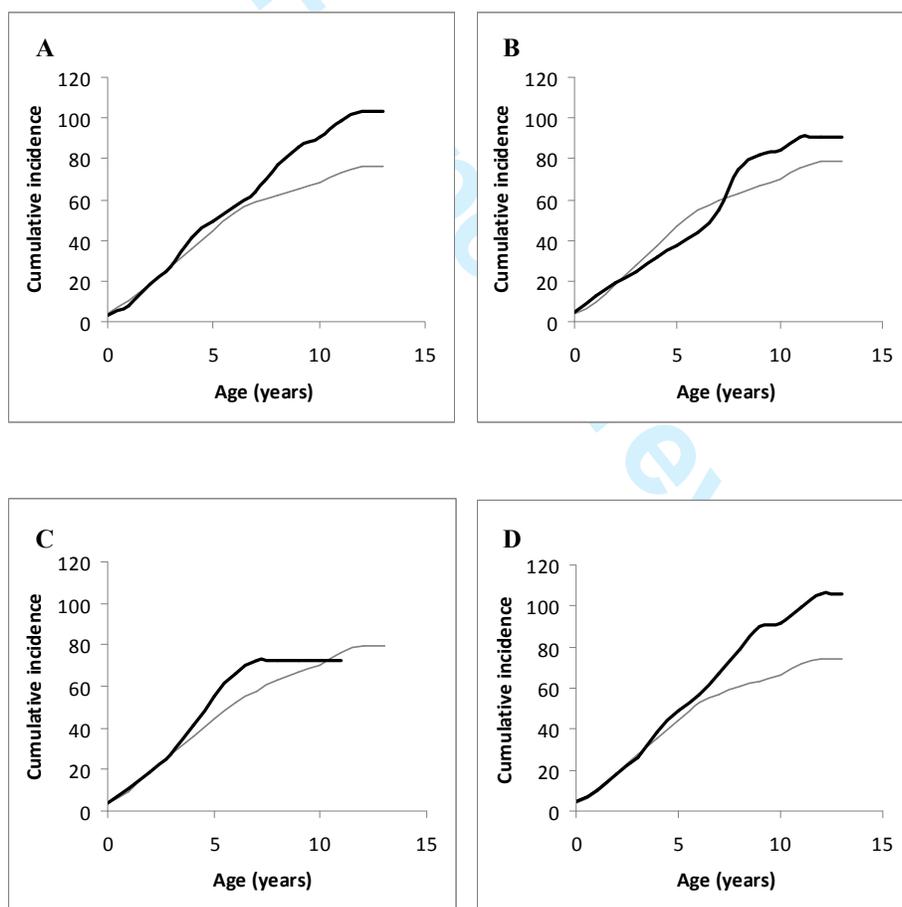
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7 **Figure 1** Annual incidence rate of clinical *P. falciparum* episodes per 100 children (bold
8 line). In order to overcome the fluctuations of the annual incidence rate, we fit second (dotted
9 line), third (dashed line) and fourth (solid line) degree polynomial trend lines to the data (bold
10 line). The corresponding R-squared values are 0.70, 0.91 and 0.99 respectively indicating an
11 accurate fit for third and fourth order polynomials. The inflexion on these two trend lines
12 indicates the onset of acquisition of clinical immunity at approximately 3 to 4 years of age.
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7 **Figure 2** Mean cumulative number of *P. falciparum* clinical episodes with age for the (A)
8 Asthma, (B) Rhinoconjunctivitis and (C) Atopic dermatitis classes and overall Atopy class
9 (D) (bold lines) compared to individuals without symptoms of each respective allergy type
10 (thin lines). In all cases moderate and severe classes are combined and compared to
11 individuals without allergy symptoms. Note there are no children older than 11 years of age
12 with Atopic dermatitis.
13
14



3. Until what age did your child breastfeed **exclusively** without ever taking other aliments (fruits, vegetables, rice, meat, fish, etc.) or liquids (powdered milk, cow or goats milk, fruit juice, water, etc.) ?
- < 6 months ₁ 6 – 12 mths ₂ 12 – 24 mths ₃ NSP ₉

AGEBREAST

Illness and vaccination : Consultation of health records of child

1. Has your child enfant had the following illnesses?

Malaria : ₀ No ₁ Yes ₉ NSP

MALAR

Tuberculosis treated : ₀ No ₁ Yes ₉ NSP

TUBTRT

Helminths (oxyures, ascaris, taenia, etc.) : ₀ No ₁ Yes ₉ NSP

HEMINTH

Amoeba : ₀ No ₁ Yes ₉ NSP

AMOEBA

Measles : ₀ No ₁ Yes ₉ NSP

MEASLES

2. Against what illnesses is your child vaccinated?

Yellow fever : ₀ No ₁ Yes ₉ NSP

VACFJ

Hepatitis B : ₀ No ₁ Yes ₉ NSP

VACHEPB

Measles : ₀ No ₁ Yes ₉ NSP

VACMEASLE

Mumps : ₀ No ₁ Yes ₉ NSP

VACMUMPS

Rubella : ₀ No ₁ Yes ₉ NSP

VACRUBEL

Tuberculosis/BCG : ₀ No ₁ Yes ₉ NSP

VACTUB

Diphtheria/Tetanus/Pertussis/Poliomyelitis : ₀ No ₁ Yes ₉ NSP

VACDTCP

Typhoid : ₀ No ₁ Yes ₉ NSP

VACTY

Meningitis : ₀ No ₁ Yes ₉ NSP

VACMENIN

Haemophilus influenzae type B (HiB) : ₀ No ₁ Yes ₉ NSP

VACHIB

Habitation :

1. Which of these animals / insects can be found in the **rooms** where your child lives (today and/or during his first year of life) ?

Dogs in rooms today : ₀ No ₁ Yes ₉ NSP

DOGTODAY

Dogs in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

DOG01YR

Cats in rooms today : ₀ No ₁ Yes ₉ NSP

CATTODAY

Cats in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

CAT01YR

Sheep in rooms today : ₀ No ₁ Yes ₉ NSP

SHEEPTODAY

Sheep in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

SHEEP01YR

Goats in rooms today : ₀ No ₁ Yes ₉ NSP

GOATODAY

Goats in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

GOA01YR

Chicken, ducks in rooms today : ₀ No ₁ Yes ₉ NSP

CHICTODAY

Chicken, ducks in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

CHIC01YR

Rodents (rats, mice, etc.) in rooms today : ₀ No ₁ Yes ₉ NSP

RODTODAY

Rodents (rats, mice, etc.) in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

ROD01YR

Cockroaches in rooms today : ₀ No ₁ Yes ₉ NSP

COCTODAY

Cockroaches in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

COC01YR

Other in rooms today : ₀ No ₁ Yes ₉ NSP

OTHTODAY

Other in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

OTH01YR

If Others, define :

..... NAMEOTH

2. Which of these animals could be in **contact** with your child **at least once per week**

(today and/or during his first year of life) ?

Contact with Dogs today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CDOGTODAY
Contact with Dogs 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CDOG01YR
Contact with Cats today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CCATODAY
Contact with Cats 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CCAT01YR
Contact with Sheep today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CSHEEPTODAY
Contact with Sheep 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CSHEEP01YR
Contact with Goats today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CGOATODAY
Contact with Goats 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CGOA01YR
Contact with Chicken, Ducks today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CCHICTODAY
Contact with Chicken, Ducks 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CCHIC01YR
Contact with donkeys, horses today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHORSTODAY
Contact with donkeys, horses 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHORS01YR
Contact with Cows, zébus today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CCOWTODAY
Contact with Cows, zébus 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CCOW01YR
Contact with Rodents (rats, mice, etc.) today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CRODTODAY
Contact with Rodents (rats, mice, etc.) 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CROD01YR
Contact with Other today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	COTHTODAY
Contact with Other 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	COTH01YR
If Others, define :		<input type="checkbox"/>	NAMEOTHC

3. Which of these aliments are usually stocked in the rooms where your child lives ?

Millet kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MIL
Sorghum kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	SORG
Maize kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MAIZ
Rice kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RICE
Wheat kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WHEA
Biscuits, pasta kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	BISCUI
Manioc (root, flour) kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MANIOC
Cashew nut, ground nut kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	NUTP
Curdled milk kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MILKCURD
Dried leaves (mint, quinquiliba, baobab, etc.) :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	LEAF
Other aliments kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHALIM
If Others, define :		<input type="checkbox"/>	NAMEOTHAL

What is the type of roofing of the rooms where your child lives (today and during the first year of life) ?

Corrugated metal roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RMETTODAY
Corrugated metal roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RMET01YR
Thatched roof today:	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RTHATDAY
Thatched roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RTHAT01YR
Wooden roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RWOOTODAY
Wooden roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RWOO01YR
Cement roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RCEMTODAY
Cement roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RCEM01YR
Plaster roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RPLATODAY
Plaster roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RPLA01YR
Other type of roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ROTHTODAY

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Other type of roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ROTH01YR
If other, define :			NAMEOTHR
4. Which of these objects are in the room where your child sleeps (today and during the first year of life) ?			
Mattress in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MATRTODAY
Mattress in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MATR01YR
Bednet in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	BEDNTODAY
Bednet in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	BEDN01YR
Wardrobe in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WARDTODAY
Wardrobe in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WARD01YR
Chest, trunk in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHESTODAY
Chest, trunk in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHES01YR
Table in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	TABPTODAY
Table in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	TABP01YR
Chair in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHPTODAY
Chair in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHA01YR
Carpet, rug in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CARPTODAY
Carpet, rug in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CARP01YR
Matting in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MATPTODAY
Matting in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MATP01YR
Curtains in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CURTTODAY
Curtains in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CURT01YR
Malagasy fire in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FIRTTODAY
Malagasy fire in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FIR01YR
Other objects in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHOBTTODAY
Other objects in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHOB01YR
If other, define :			NAMEOTHOB
5. On what type of bedding does your child sleep (today and during the first year of life) ?			
Foam mattress today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FMATRTODAY
Foam mattress 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FMATR01YR
Plant fibre mattress (straw, etc.) today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLFMATRTODAY
Plant fibre mattress (straw, etc.) 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLFMATR01YR
Wool mattress today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WOMATRTODAY
Wool mattress 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WOMATR01YR
Feather mattress today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FEATHMTODAY
Feather mattress 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FEATHM01YR
Plastic matting today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLMATTTODAY
Plastic matting 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLMAT01YR
Plant fibre matting (straw, etc.) today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLFMATTTODAY
Plant fibre matting (straw, etc.) 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLFMAT01YR
Other type of bedding today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHBEDTTODAY
Other type of bedding 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHBED01YR
If other, define :			NOMAUTLI
6. Does your child sleep on a pillow ?	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PILLOW
If No, go to question 8			

If **Yes**, what type of pillow is it ?

- Foam : No Yes NSP PILLF
- Synthetic fibres: No Yes NSP PILLSYN
- Plant fibres (straw, etc.) : No Yes NSP PILLPLF
- Feather : No Yes NSP PILLFEATH
- Other type of pillow : No Yes NSP OTHPILL

If other, define :..... NAMEOTHPILL

7. Do people smoke in the room where your child lives ?

- Today : No Yes NSP SMOKTODAY
- From 0-1yr : No Yes NSP SMOK01YR
- During the pregnancy of the mother : No Yes NSP SMOKPREG

8. What type of heating and lighting are used in the rooms where your child lives ?

- Heating and lighting by charcoal : No Yes NSP CHELCHAR
- Heating and lighting by wood : No Yes NSP CHELWOO
- Lighting by candle : No Yes NSP LCAND
- Lighting by petrol lamp : No Yes NSP LLAMP
- Lighting by flash light : No Yes NSP LTORCH
- Lighting by solar : No Yes NSP LSOLAR
- Other types of heating and lighting: No Yes NSP OTHHEL

If other, define :..... NAMEOTHHEL

9. Which of the following products are used or stocked in the rooms where you child lives ?

- Insecticide (type Yotox, spirales, etc.) : No Yes NSP INSECTIC
- Deodorants (aerosols) : No Yes NSP DEODORA
- Incense : No Yes NSP INCENSE
- Detergents (type Cotel, etc.) : No Yes NSP DETERGEN
- Petrol, diesel : No Yes NSP PETROL
- Other types of products : No Yes NSP OTHPROD

If other, define :..... NAMEOTHPR

Diet :

1. Has your child had **diarrhoea without fever** or abdominal pains (colic)

following introduction of non-maternal milk in his diet (cow or goat's milk, milk powder) : No Yes NSP DIARINT

after a few months of consuming **non-maternal** (cow or goat's milk, milk powder) : No Yes NSP DIARMONTH

2. Currently, how many times, on average, does your child eat the following aliments ?

The consumption of certain aliments is seasonal.

- Meat : Never <1times/week 1-2 times/week ≥1times/day CONSMEAT
- Fish : Never <1times/week 1-2 times/week ≥1times/day CONSFISH
- Egg : Never <1times/week 1-2 times/week ≥1times/day CONSEGG
- Milk (liquid, powder, curdled) : Never <1times/week 1-2 times/week ≥1times/day CONSMILK
- Banana : Never <1times/week 1-2 times/week ≥1times/day CONSBANA
- Mango : Never <1times/week 1-2 times/week ≥1times/day CONSMANG
- Melon : Never <1times/week 1-2 times/week ≥1times/day CONSMELON

1	Orange, lime :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSORAN	
2	Potatoes, sweet potatoes :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSPOT	
3	Vegetables :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSVEG	
4	Millet :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSMIL	
5	Sorghum :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSSORG	
6	Maize :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSMSAIS	
7	Rice :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSRICE	
8	Wheat (bread, pasta) :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSWHEA	
9	Nuts (Cashew, ground nut) :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSNUT	
10	Prawns, dried oysters :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSPRAWN	
11	Flavouring cubes Maggi :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSCUBE	
12	Other :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	OTHALCON	
13		If other, define :					<input type="checkbox"/>	NAMEOTHAL

HISTORICAL SYMPTOMATOLOGY OF ALLERGIC REACTIONS

Asthma :

1. Has a doctor or nurse **already** said that you child has asthma ?

₀ No ₁ Yes ₉ NSP

ASTHMA

2. Has your child already breathed noisily or had whistling in his chest whilst breathing

₀ No ₁ Yes ₉ NSP

WHISTLING

If **No**, go directly to question 6

3. During his first two years of life, has your child already breathed noisily or had whistling in his chest whilst breathing ?

₀ No ₁ Yes ₉ NSP

WHISTL2YR

If **No**, go directly to question 6

If **Yes**, how many times (before 2 years of age) ?

₁ 1time ₂ 2times ₃ ≥3times ₉ NSP

NBWHIS2YR

Between the last two **ramadans**, has your child already breathed noisily or had whistling in his chest whilst breathing ?

₀ No ₁ Yes ₉ NSP

WHISTL2RA

If **No**, go directly to question 5

If **Yes**, at which moment of the year ?

Rainy season : ₀ No ₁ Yes ₉ NSP

WHISTLRS

Dry season : ₀ No ₁ Yes ₉ NSP

WHISTLDS

Harvest time : ₀ No ₁ Yes ₉ NSP

WHISTLHT

Has the noisy breathing of your child been such that it has prevented him from talking normally?

₀ No ₁ Yes ₉ NSP

PREVTALK

Has your child already had a rasping cough at night that prevents him from sleeping normally ?

₀ No ₁ Yes ₉ NSP

TOUSECHE

Rhinitis and allergic conjunctivitis:

1. Has your child **already had** problems of a runny nose, or a sensation of a blocked nose, or an itchy nose, or sneezing, or loss of the sense of smell **for more than a week**,

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irrespective of the frequency of these episodes? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHIN1WEEK
2. Has your child already had problems of a runny nose, or a sensation of a blocked nose, or an itchy nose, or sneezing, or loss of the sense of smell more than 5 times in one year , irrespective of the frequency of these episodes? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHIN5FAN
Between the last two ramadans , has your child already had problems of a runny nose, or a sensation of a blocked nose, or an itchy nose, or sneezing, or loss of the sense of smell ? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHIN2RAM
If No , go to question 4		
If Yes , at what moment of the year ?		
Rainy season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHINRS
Dry season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHINDS
Harvest time : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHINHT
3. Has your child already had watery eyes, or itchy eyes, or an allergic limbo-conjunctivitis? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJALER
If No , go directly to question 1 in the section Eczema		
Has your child had, between the last two ramadans , watery eyes, or itchy eyes, or an allergic limbo-conjunctivitis? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJ2RAM
If No , go directly to question 5		
If Yes , at what moment of the year ?		
Rainy season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJRS
Dry season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJDS
Harvest time : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJHT
<u>Eczéma :</u>		
Has your child already had skin problems with dry patches or seeping cracked patches and itching ? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEMA
If No , the questionnaire has finished.		
Between the last two ramadans , has your child had skin problems with dry patches or seeping cracked patches and itching ?? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZE2RAM
If No , go directly to question 3		
If Yes , at what moment of the year ?		
Rainy season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEMARS
Dry season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEMADS
Harvest time : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEMAHT
1. Have these skin problems affected different parts of the body of your child ?		
Scalp : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZESCALP
Face : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEFAC
Around the eyes and ears : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	
Armpits : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEEYEAR

STROBE Statement—checklist of items included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses

Continued on next page

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure <i>Cross-sectional study</i> —Report numbers of outcome events or summary measures
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

Discussion

Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based
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*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.



Asthma and atopic dermatitis are associated with increased risk of clinical Plasmodium falciparum malaria

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Manuscripts

Asthma and atopic dermatitis are associated with increased risk of clinical *Plasmodium falciparum* malaria

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Article summary

Article focus

- Genetic studies suggest a link between susceptibility to allergy and malaria in Africa
- We hypothesize that atopy increases susceptibility to malaria

Key messages

- Results demonstrate an association between asthma, atopic dermatitis and susceptibility to clinical *P. falciparum* episodes.
- Genetic pre-disposition to asthma or atopic dermatitis impairs the acquisition of clinical immunity to malaria.
- Administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Strengths and limitations

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association.

Abstract

Objectives: To assess the impact of atopy and allergy on the risk of clinical malaria.

Design: A clinical and immunological allergy cross-sectional survey in a birth cohort of 175 children from 1 month to 14 years of age followed for up to 15 years in a longitudinal open cohort study of malaria in Senegal. Malaria incidence data were available for 143 of these children (aged 4 months to 14 years of age) for up to 15 years. Mixed model regression analysis was used to determine the impact of allergy status on malaria incidence, adjusting for age, sickle cell trait and force of infection.

Main outcome measures: Asthma, allergic rhinoconjunctivitis and atopic dermatitis status, the number of clinical *Plasmodium falciparum* malaria episodes since birth and associated parasite density.

Results: Twelve percent of the children were classified as asthmatic and ten percent as having atopic dermatitis. These groups had respectively a two-fold (OR 2.12 95% confidence intervals 1.46 to 3.08; $P= 8 \times 10^{-5}$) and three-fold (OR 3.15, 1.56 to 6.33; $P= 1.3 \times 10^{-3}$) increase in the risk of clinical *P. falciparum* malaria once older than the age of peak incidence of clinical malaria (3 to 4 years of age). They also presented with higher *P. falciparum* parasite densities (Asthma: mean 105.3 parasites/ μ L \pm SE 41.0 vs. 51.3 \pm 9.7; $P= 6.2 \times 10^{-3}$; Atopic dermatitis: 135.4 \pm 70.7 vs. 52.3 \pm 11.0; $P=0.014$). There was no effect of allergy on the number of non-malaria clinical presentations. Individuals with allergic rhinoconjunctivitis did not have an increased risk of clinical malaria nor any difference in parasite densities.

Conclusion: These results demonstrate that asthma and atopic dermatitis delay the development of clinical immunity to *P. falciparum*. Despite the encouraging decrease in malaria incidence rates in Africa, a significant concern is the extent to which the increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines, administration to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Introduction

The World Allergy Organization estimates that 40% of the world's population is concerned by allergic diseases.¹ In developing countries where *Plasmodium falciparum* malaria is endemic, prevalence of allergy is significantly lower, but is on the increase.² Orientation of the immune response towards a Th1 profile is crucial for immunity to intracellular pathogens,³ whereas orientation towards a Th2 profile drives immunity to extracellular pathogens and antigens resulting in class switching giving rise to IgE-producing B cells.⁴ An important role of the Th1/Th2 balance in the development of clinical malaria following infection by *P. falciparum* has been suggested by numerous studies.⁵⁻⁷ It has been suggested that the Th2 bias induced by *P. falciparum* may exacerbate allergy.⁸ Likewise, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*. However, the interplay between infectious agents and allergy is unclear. On the one hand, for example, severe respiratory syncytial virus infection in infants increased the risk of allergic rhinoconjunctivitis and allergic asthma.^{9,10} On the other hand, measles,¹¹ hepatitis A¹² and tuberculosis¹³ seemingly reduce atopy. Although, an atopic condition can increase incidence of disease, such as the case for the skin commensal *Staphylococcus aureus* in patients with atopic dermatitis,¹⁴ an atopic tendency *per se* does not generally lead to increased illness from infectious agents.

Genome wide studies have identified chromosomal regions linked to clinical malaria, all of which overlap with those previously identified to be involved in atopic dermatitis, asthma, atopy and IgE levels,¹⁵⁻¹⁷ suggesting that common mechanisms may be involved in both pathologies.¹⁸ Chromosomal region 5q31 that has been repeatedly shown to be associated with control of parasite density and contains a cluster of cytokines, among which IL12B has been previously associated with psoriasis.¹⁹ The other regions, 13q13-q22, 5p15-p13 and 12q21-q23, contain genes involved in innate immunity, notably the interleukin 7 receptor, and several involved in tumour necrosis factor synthesis [C1q and tumour necrosis factor related protein 3 (C1QTNF3)] and a gene involved in the complement system (C9).¹⁸

Several additional lines of evidence support the concept that susceptibility to malaria and atopy may be related to similar immunological defects. In Ethiopia, a history of malaria was associated with atopy.²⁰ A mouse model for human atopic disease was found to be very susceptible to murine malaria and a major locus for atopic disease mapped close to the

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3 region controlling parasite density.²¹ This region contains several candidate genes that have
4 effects on T-cell function.²¹
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7 Moreover, a direct effect of histamine in the malaria pathogenesis has been found using
8 genetic and pharmacological approaches²² and increased levels of histamine are associated
9 with the severity of disease in humans infected with *P. falciparum* and in animal malaria
10 models.^{23,24}
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14 To test the hypothesis that allergy impacts upon clinical *P. falciparum* malaria, we performed
15 a clinical allergy cross-sectional study in the family-based longitudinal cohort from Senegal
16 previously used for the genome linkage study¹⁸ and analysed the impact of asthma, atopic
17 dermatitis, allergic rhinoconjunctivitis on the incidence of clinical *P. falciparum* episodes and
18 the maximum parasite density during each episode.
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24 25 26 **Methods**

27 28 **Population and outcome data**

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31 The malaria research program conducted in Dielmo village in Senegal has been ongoing
32 since 1990 as described elsewhere.²⁵ In brief, between 1990 and 2008, a longitudinal study
33 involving the inhabitants of the village of Dielmo, Senegal, was carried out to identify all
34 episodes of fever. The study design included daily medical surveillance with systematic blood
35 testing of individuals with fever and examination of 200 oil-immersion fields on a thick blood
36 film for malaria parasites (about 0.5 µL of blood). Each individual was given a unique
37 identification code and details of family ties, occupation, and precise place of residence were
38 recorded on detailed maps of each household with the location of each bedroom. All
39 households were visited daily, absenteeism recorded, and the presence of fever or other
40 symptoms assessed. We systematically recorded body temperature at home three times a
41 week (every second day) in children younger than 5 years, and in older children and adults in
42 cases of suspected fever or fever-related symptoms. In cases of fever or other symptoms,
43 blood testing was done at the dispensary by finger prick, and we provided detailed medical
44 examination and specific treatment. Parasitologically confirmed clinical malaria episodes
45 were treated according to national guidelines. From 1990 to 2008, four different drug
46 regimens were implemented: Quinine from 1990 to 1994, Chloroquine from 1995 to 2003,
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3 Fansidar (sulfadoxine-pyrimethamine) from 2004 to mid-2006 and Artemisinin-based
4 combination therapy (ACT; Amodiaquine- sulfadoxine-pyrimethamine) from mid-2006 to
5 2008.
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8 Parasite positivity was established as follows. Thick blood films were prepared and stained
9 by 3% Giemsa stain. Blood films were examined under an oil immersion objective at x1000
10 magnification by the trained laboratory technicians and 200 thick film fields were examined
11 to count the number of asexual and gametocyte parasite stages. Asexual parasite densities
12 (per μL) were calculated by establishing the ratio of parasites to white blood cells and then
13 multiplying the parasite count by 8,000, the average white blood cell count per μL of blood.
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19 Malaria transmission in Dielmo is intense and perennial. We conducted a cross-sectional
20 survey to estimate the prevalence of symptoms related to allergic diseases among 175
21 children aged from 1 month to 14 years old who were born during the malaria research
22 program.
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27 Both the longitudinal and cross-sectional surveys were approved by the Ministry of Health of
28 Senegal. Informed consent of the volunteers is renewed every year. More specifically for the
29 cross-sectional survey, after informing about the procedures and the purpose of the study,
30 written informed consent was obtained from parents or guardians of children either by
31 signature or by thumbprint on a voluntary consent form written in both French and Wolof,
32 the main local language. Consent was obtained in the presence of the school director, an
33 independent witness.
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40 The family structure (pedigree) was available after a demographic census performed for
41 every volunteer at his adhesion in the project. A verbal interview of mothers or key
42 representatives of the household was used to obtain information on genetic relationships
43 between studied individuals, their children, their parents, and to identify genetic links
44 among the population. The total pedigree comprised 828 individuals, including absent or
45 dead relatives, composed of ten independent families that can be sub-divided into 206
46 nuclear families (father – mother couples with at least one child) with an average of 3.6
47 children each. Genetically related nuclear families occur because of multiple marriages and
48 marriages among related individuals. Previous typing with microsatellites has enabled the
49 construction of a pedigree based on Identity-by-Descent using MERLIN.^{18,26} The mean
50 coefficient of inbreeding is 0.0008. Newborns since this original genetic analysis were added
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3 to the family of the parents in question. The 143 children, with both allergy and malaria
4 data, belonged to 61 nuclear families and comprised 30 singletons, 102 siblings and 11 half-
5 sibs (yielding 55 half-sib pairs). The mean genetic relatedness (by pedigree) of the 143
6 children is 0.0114 (range: 0.0013 to 0.022).
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10 *P. falciparum clinical episodes*

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12 *P. falciparum* malaria clinical episode phenotypes analysed were: (i) clinical *P. falciparum*
13 infections treated with anti-malarial therapy and (ii) the highest parasite density during the
14 *P. falciparum* clinical episode. A clinical *P. falciparum* episode was defined as a clinical
15 presentation with fever (axillary temperature $\geq 37.5^{\circ}\text{C}$) and/or other clinical signs suggestive
16 of malaria associated with a thick blood smear positive for *P. falciparum* and that was
17 treated with anti-malarial therapy. Repeated clinical malaria presentations within 15
18 consecutive days were not considered to be independent and were excluded from the
19 analyses, unless there was a negative thick blood smear between two clinical presentations.
20 We also excluded observations in any trimester for which the individual was not present for
21 at least one third of the time.
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31 We calculated the quarterly incidence rate of clinical *P. falciparum* episodes in children
32 below the age of 15 years as the ratio of the total number of clinical *P. falciparum* episodes
33 during the trimester divided by the total number of person-trimesters surveyed. Incidence
34 rate is expressed as cases per 100 person-trimesters (see Supplementary Figure S1). This
35 rate was used in the analysis to approximate the force of infection (exposure level) within
36 the targeted population at the time of a given clinical *P. falciparum* episode.
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42 The total number of clinical presentations per trimester that were not attributable to *P.*
43 *falciparum* was tabulated. Repeated non-malaria presentations within seven consecutive
44 days were not considered to be independent and were excluded.
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48 *Allergic diseases and atopic status*

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50 The International Study of Asthma and Allergies in Childhood (ISAAC) diagnostic criteria have
51 been shown to be reproducible, adequate and able to discriminate children with allergic
52 diseases in different areas of the world.² The standardized ISAAC questionnaire originally
53 written in English was translated into French in compliance with ISAAC guidelines²⁷, adapting
54 it to the usual local customs following advice from local clinicians and paediatric
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3 allergologists (Acknowledgements and Technical Appendix). The adequacy and reliability of
4 the translated questionnaire had been previously confirmed by a pilot study on 30 randomly
5 selected children in the same community. The questionnaire was completed by specially
6 trained health workers during an oral interview conducted in Wolof with children and their
7 mothers or guardians.
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11 To assess the prevalence of allergic diseases in children, we used the positive and negative
12 predictive values of the ISAAC questionnaire diagnosis criteria developed for subtropical
13 countries.²⁸ Each question was scored according to the medical diagnosis of paediatricians
14 and paediatric allergologists. Positive or negative answers were thus graded on the basis of
15 symptom sensitivity, specificity, frequency, location or early onset. For each allergic disease,
16 three categories of symptom severity, *severe*, *moderate*, and *none*, were defined as follows:
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21 *Asthma – severe* symptoms if the child had “wheezing or whistling in the chest before the
22 age of two years” and “more than three times” or severe enough to “limit his/her speech”;
23 *moderate* symptoms if the child had “wheezing or whistling in the chest before the age of
24 two years” and “in the past 12 months”; and *none* otherwise.
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29 *Allergic rhinoconjunctivitis – severe* symptoms if the child had “sneezing, runny or stuffy nose
30 in the past 12 months” and “more than five times a year”, and “itchy, watery eyes or tropical
31 endemic limboconjunctivitis (TELC) in the past 12 months”; *moderate* symptoms if the child
32 had “sneezing, runny or stuffy nose in the past 12 months”, and “itchy, watery eyes or TELC
33 in the past 12 months”; and *none* otherwise.
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38 *Atopic dermatitis – severe* symptoms if the child had “scaly or exudating, crusted and pruritic
39 patches in the past 12 months” and “affecting any of the following characteristic areas: face,
40 around the ears or eyes, folds of armpits or elbows or groin, behind the knees, under the
41 buttocks”, and “onset of symptoms before the age of two years”; *moderate* symptoms if the
42 child had “scaly or exudating, crusted and pruritic patches in the past 12 months” and
43 “affecting any of characteristic areas (see above)”, and “onset of symptoms before the age
44 of four years”; and *none* otherwise.
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49 The inter-relationships between variables reflecting the severity of symptoms of the three
50 allergic diseases were used to identify children at high risk of atopy. The *high probability*
51 group was defined by the prevalence of at least one of any *severe* symptoms or two of any
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3 moderate symptoms. The *probable* group was defined as those with *moderate* symptoms
4 from one of the three allergic diseases and remaining children were classified in the *unlikely*
5 group.
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8 *Helminths*

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10 Helminthic infections are common in this region and are known to modify the clinical course
11 and outcome of both allergic diseases and malaria.^{29,30} We therefore carried out a helminth
12 survey for 91 individuals present during the cross-sectional survey. Diagnosis was performed
13 by stool examination by microscope and by the Kato technique to search for the presence of
14 *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*),
15 whipworm (*Trichuris trichiuria*), *Schistosoma mansoni*, and *Strongyloides stercoralis*.
16 Examination for pinworms (*Enterobius vermicularis*) was performed by the anal scotch-test.
17 An anti-helminthic treatment was proposed for all infested individuals.
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25 *Immunoglobulin E titres*

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27 Specific IgE titres were measured by ELISA as previously described.³¹ A panel of allergens of
28 potential pertinence to the three classes of allergy was used: (i) Salivary gland extracts (SGE)
29 of two mosquito species present in the study cohorts, *Aedes aegypti* and *Anopheles gambiae*
30 *sensu stricto*, and (ii) *P. falciparum* parasite extract were prepared as previously described³¹;
31 (iii) House dust mite spp. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*;
32 (iv) a mix of pollen allergens from five ubiquitous gramineae spp. [Cock's-foot (*Dactylis*
33 *glomerata*), Timothy grass (*Phleum pratense*), Sweet Vernal grass (*Anthoxanthum*
34 *odoratum*), Perennial ryegrass (*Lolium perenne*), Kentucky Bluegrass (*Poa pratensis*)] (all
35 from Stallergenes, France).
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44 **Statistical analysis**

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46 Statistical analyses were performed using R version 2.12.0 (The R Foundation for Statistical
47 Computing, Vienna, Austria). To address the effect of allergic status on the risk of clinical *P.*
48 *falciparum* episodes, we performed Generalized Linear Mixed Models (GLMM) extended to
49 pedigree data using the *pedigreemm* package for R to account for the non-independence of
50 individuals because of family relationships, shared house and for repeated measures from
51 the same individual (Technical Appendix). Correlated individual effects due to familial
52 relationships were taken into account by using the pedigree-based genetic relatedness
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3 matrix that contains the genetic covariance among all pairs of individuals in the study cohort
4 and is calculated using the pedigree information.³² Shared house and repeated measures
5 from the same individual were modelled as random effects. All random effects were
6 assumed to be normally distributed, and conditional on these random effects, the
7 dependent variable had: (i) a Binomial distribution when the studied phenotype was the
8 occurrence of a clinical *P. falciparum* episode treated with anti-malarial therapy during a
9 trimester, (ii) a Gaussian distribution when the studied phenotype was the logarithm of the
10 maximum parasite density during a given clinical *P. falciparum* episode, and (iii) a Poisson
11 distribution when the studied phenotype was the number of non-malaria episodes per
12 trimester. The effects of allergy disease classes on these dependent variables were modelled
13 as fixed effects. Allergy classes were reduced to two levels, *Severe* or *moderate* vs. *none* for
14 analyses of asthma, atopic dermatitis and allergic rhinoconjunctivitis and *high probability* vs.
15 *probable* and *unlikely* for atopic tendency. Co-variables included sickle cell trait³¹, gender,
16 number of days present on site during the trimester, trimestrial incidence of *P. falciparum*
17 and age. Age was initially analysed as a continuous covariate. To assess the age-specific
18 effect of allergy, age was categorised into two levels (<3.5 years of age and ≥3.5 years of
19 age, based on the age of peak clinical incidence) and allergy class was nested within age
20 class. The age threshold was varied from 1.5 years to 5.5 years of age and the data re-
21 analysed to assess at which age there was the strongest effect. The association of allergy
22 classes with IgE levels was analysed by box-cox transforming the data and fitting a GLMM
23 with a normal distribution.
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43 Results

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45 Of the 205 eligible children aged under 15 years involved in the family-based longitudinal
46 study, 175 (85.4 %) participated in the cross-sectional survey to assess the prevalence of
47 related symptoms of allergic diseases. All eligible children present at the time of the survey
48 were included; no explicit refusal to participate was recorded. The study cohort was aged
49 from 1 month to 14 years 11 months. The sex-ratio (male/female) was 0.94.
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54 From 1994 until 2008, 143 of the children participating in the cross-sectional survey were
55 present for at least 31 days in any trimester during the study period generating a total of
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3 3,093 person-trimesters of presence (Supplementary Table S1). There were 2,065 treated *P.*
4 *falciparum* clinical episodes (median 11, range 0-47)(Supplementary Table S2). The age peak
5 of incidence of *P. falciparum* episodes occurred at 3 to 4 years of age (Figure 1). There were
6 1,868 non-malaria episodes (median 12, range 0-37) (Table S2). These non-malaria clinical
7 presentations were associated with headache (38 %), chills (32 %), cough (13 %), vomiting
8 (11 %) and diarrhoea (6 %).

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14 The prevalence of moderate or severe asthma symptoms was respectively 2.3 % and 10.3 %
15 (Table 1). The prevalence of moderate or severe allergic rhinoconjunctivitis symptoms was
16 respectively 6.3 % and 10.3 %. The prevalence of moderate or severe atopic dermatitis
17 symptoms was respectively 6.3 % and 2.9 %. On the basis of symptom severity, an atopic
18 tendency was estimated to be unlikely for 68.0 %, probable for 9.1 % and highly probable for
19 22.9 % of the 175 children. The frequency of each allergy class in children for whom malaria
20 data were available is shown in Table S1.

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27 The risk of treated clinical *P. falciparum* infections was higher for children with high
28 probability of atopy (OR 1.65, 95% confidence intervals 1.20 to 2.26; P=0.002) (Table 2), after
29 adjusting for age, sickle cell trait and the exposure level. Gender was not found to be
30 significant. Analysing the impact of atopy in children younger and older than the peak age of
31 clinical incidence (3 to 4 years old), revealed that atopy increased the risk of *P. falciparum*
32 episodes in children at an age greater than 3.5 years (OR 2.02, 1.39 to 2.93; P=2x10⁻⁴), but
33 not in children of age prior to the peak clinical incidence (OR 1.38, 0.92 to 2.08; P=0.124)
34 (Table 2). This increased risk resulted in an ever increasing cumulative number of *P.*
35 *falciparum* episodes with age beyond that of peak clinical incidence (Figure 2. See
36 supplementary Figure S2 for model predictions for comparison).

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45 Analysis by allergy category revealed that asthma (severe or moderate) increases the risk of
46 *P. falciparum* episodes (OR 2.12, 1.46 to 3.08; P= 8 x10⁻⁵) and this again only in children of
47 age greater than 3.5 years old (OR 2.33, 1.50 to 3.61; P= 1.5 x10⁻⁴). Atopic dermatitis
48 increased the risk of clinical malaria in children older (OR 3.15, 1.56 to 6.33; P= 1.3 x10⁻³) but
49 not younger than 3.5 years of age (Table 2). Allergic rhinoconjunctivitis was not associated
50 with increased risk of clinical malaria at any age (Table 2). The impact of atopy, asthma and
51 atopic dermatitis can be clearly seen in the ever-increasing number of cumulative *P.*
52 *falciparum* episodes beyond the age of the onset of clinical immunity in the population, 3.5
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3 years of age (Figure 2). There is no difference in the number of clinical malaria episodes prior
4 to this age in individuals with or without an allergic condition. Analysis using different age
5 thresholds (from 1.5 to 5.5 years of age) revealed similar OR for thresholds of 2.5, 3.5 and
6 4.5 years of age. The maximum OR for increased malaria occurred in children older than 4.5
7 years of age and with atopy or atopic dermatitis, whereas for the asthma group it occurred
8 in children after 3.5 years of age (Supplementary Table S3).

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14 There was no impact of any allergic disease on the number of non-malaria episodes by
15 trimester (Supplementary Table S4).

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18 The impact of atopy, asthma and atopic dermatitis on the maximum *P. falciparum* parasite
19 density during a given clinical malaria episode mirrored that of the risk of *P. falciparum*
20 episodes. Parasite density was significantly higher for children with allergic disease older
21 than 3.5 years of age (Table 3 and supplementary Figure S3 for residuals of the fitted model).
22 Allergic rhinoconjunctivitis had no impact on the parasite density (Table 3). Analysis using
23 different age thresholds yielded the same pattern as seen with the number of clinical
24 episodes (Table S3).

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31 Individuals with moderate or severe symptoms of atopic dermatitis had significantly higher
32 specific IgE titres against *Ae. aegypti* (P=0.004) and *An. gambiae* SGE (P<0.001). There were
33 no detectable specific anti-*P. falciparum* IgE. Individuals with moderate or severe symptoms
34 of allergic rhinoconjunctivitis did not have significantly higher IgE titres against the tested
35 graminiae (P=0.28), although titres decreased with age (P=0.035). There was also no effect of
36 asthma on IgE titres against the house dust mite spp. tested (*D. farinae* P=0.60 & *D.*
37 *pteronysinus* P=0.27).

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44 Only five individuals were infested with helminths (two *Ancylostoma*, one *Strongyloides*, one
45 *Trichuris* and one *Enterobius*).

46 47 48 49 **Discussion**

50 51 52 **Principal findings**

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55 Establishing the allergic status of children up to the age of 15 years old followed for malaria
56 since birth, revealed an association of asthma and atopic dermatitis with susceptibility to
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3 clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with
4 these allergic conditions occurred after the peak clinical incidence of disease in the
5 population, suggesting that they delay the development of clinical immunity to malaria.
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8 9 **Strengths and weaknesses of the study**

10 The major strength of this study is the complete knowledge of the number of clinical *P.*
11 *falciparum* malaria episodes each individual has had since birth and the exposure level per
12 trimester over the 15 years covering the birth cohort. No other study has such detailed
13 information for such a length of time. The major weakness of the study is the relatively small
14 sample size, which would have reduced power to detect an association. In addition, although
15 allergy diagnosis for children under 2 years of age is not considered reliable, there were only
16 15 individuals under 2 at the time of the allergy study of the 143 for whom malaria and
17 allergy data were available.
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25 26 **Meaning of the study**

27 Under intense malaria transmission, after repeated exposure to the parasite, children
28 develop a clinical immunity³³, whereby they tolerate elevated parasite densities without
29 showing clinical symptoms. In this cohort, the population mean onset of clinical immunity
30 occurred at 3 to 4 years of age. Although clinical immunity is accompanied by a reduction in
31 parasite density, effective anti-parasite immunity develops much more slowly³⁴ with
32 individuals achieving a state of premunition, whereby they maintain low-grade parasite
33 densities in an asymptomatic state.³⁵ We show here that children with clinically defined
34 asthma or atopic dermatitis had a two to three-fold increase in the risk of presenting with *P.*
35 *falciparum* malaria episodes requiring treatment once passing the age of peak clinical
36 incidence. They also had higher parasite density during clinical episodes, suggesting a
37 reduced ability to control parasite replication. The observed increase in clinical incidence of
38 malaria in patients with asthma or atopic dermatitis is not likely to be the result of increased
39 frailty of such individuals; these individuals did not come more frequently to the clinic with
40 non-malaria symptoms. Our previous genome linkage study identifying chromosomal
41 regions¹⁸ associated with malaria that overlap with those previously shown to be linked to
42 asthma/atopy suggests that there may be a shared genetic basis to these pathologies rather
43 than any causative effect of one on the other. This is consistent with the increased
44 susceptibility to malaria of mouse atopic models.²¹
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Comparison with other studies

A previous study found that a history of malaria (yes/no) increased risk of atopic dermatitis in 306 cases compared to 426 controls as characterized using the ISAAC questionnaire.²⁰ The only other epidemiological study that has previously examined the link between malaria and atopy³⁶ also interpreted the result from the perspective of the impact of malaria on atopy. They examined the re-infection rate with *P. falciparum* over a 5-year period in 91 children that were subsequently classified as atopic or not using skin prick tests (SPT) with house dust mite antigen. Their conclusion was that, as with measles¹¹ and tuberculosis¹³, malaria infection reduces atopy. However, the study lacked previous infection data since birth of the participating individuals and focussed on atopy as determined by SPT against a single allergen. The case-control study of atopic dermatitis risk factors cited above found no overall association between allergen skin sensitization and atopic dermatitis. We also found no evidence of increased IgE titres against house dust mites in the asthmatic or atopic dermatitis groups or against grass pollen in individuals with allergic rhinoconjunctivitis. Such differences likely reflect the different IgE reactivity profiles due to differences in allergen exposure in Africa.³⁷ There was no evidence of anti-parasite IgE in this cohort of children. We previously showed that circulating anti-parasite IgE titres were strongly positively correlated with anti-mosquito saliva IgE, but became undetectable following malaria exposure, potentially being bound to effector cells.³¹ Only mosquito saliva, a known major local allergen, induced a specific IgE response at significantly higher titres in individuals with atopic dermatitis.

Although the immune effectors of clinical immunity are still poorly defined, there is strong evidence that acquired anti-parasite immunity is IgG-dependent³⁸ and cytophilic immunoglobulins (IgG1 & IgG3), which are capable of eliminating the parasites by opsonisation and/or by Antibody Dependent Cellular Immunity play an important role in premunition.³⁵ The higher parasite density during symptomatic episodes observed in the asthma group suggests impaired development of acquired immunity. Impaired acquisition of immunity to malaria in children with asthma or atopic dermatitis may stem from their imbalanced Th1/Th2 response. Indeed, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*. Dendritic cells that are oriented to a Th2 phenotype are more susceptible to orient the acquired immune response towards a Th2

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3 profile.³⁹ Orientation of the immune response towards a Th2 profile by asthma or atopic
4 dermatitis would result in a poor Th1 response (and hence development of protective IgG
5 immunoglobulins), considered to be the dominant arm of the immune response enabling
6 resistance to infectious disease in children.⁴⁰
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10 Many studies have revealed an important role of histamine, a key downstream effector
11 molecule in allergic reaction, in the outcome of a malaria parasite infection.^{22-24,41-44}

12 Moreover, reports indicate that components of the innate immune system, including
13 eosinophils, basophils, and mast cells (MCs), could play important roles in the pathogenesis
14 of malaria.⁴¹ Increased levels of histamine in plasma and tissue, derived from basophils and
15 MCs, notably following stimulation by IgE through the high affinity receptor FcεR1, are
16 associated with the severity of disease in humans infected with *P. falciparum* and in animal
17 malaria models.^{23,24} Chlorpheniramine, a H1 agonist reversed resistance to chloroquine and
18 amodiaquine both *in vivo* and *in vitro*.⁴² Moreover, astemizole, another H1 agonist, was
19 identified as an anti-malarial agent in a clinical drug library screen.⁴³ Finally, *P. falciparum*
20 produces translationally controlled tumor protein, which is a homolog of the mammalian
21 histamine-releasing factor that causes histamine release from human basophils.⁴⁴
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32 Further research

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34 Our results provide the first birth cohort study addressing the link between malaria and
35 allergic diseases. They contribute to a growing body of evidence that the pathologies are
36 related. ISAAC has revealed a steady but significant increase in prevalence rates of asthma
37 and allergic diseases in Africa. Whilst the majority of studies have focused on large cities,
38 there is increasing urbanization throughout Africa, as well as improved access to primary
39 health care in many areas. A key concern for ISAAC is the extent to which such societal
40 evolution will result in an increase in allergic diseases. Increased urbanization in sub-Saharan
41 Africa is changing the epidemiology of malaria and although resulting in a decrease in risk,
42 will result in more severe clinical malaria in older individuals.^{45,46} Moreover, a large
43 consumption of anti-malarial drugs in the urban areas provides substantial drug pressure
44 fostering, the selection of drug-resistant parasites. Despite the encouraging recent decrease
45 in malaria incidence rates, even in rural areas, an additional significant concern is the extent
46 to which such an increase in allergy will exacerbate the burden of malaria. Given the
47 demonstrated anti-parasitic effect of anti-histamines⁴⁷, administration of anti-histamines to
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3 atopic children will likely reduce the burden of clinical malaria in these children, increase the
4 efficacy of first-line treatment anti-malarials⁴⁸ and alleviate the non-infectious consequences
5 of atopy. Clinical intervention studies should be envisaged.
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8 9 **What is already known on this topic**

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11 There are several reports of the beneficial effects of anti-histamines for malaria
12 chemoprophylaxis^{22-24,47} as well as our previous work¹⁸ showing that chromosomal regions
13 associated with malaria are also linked to allergy and atopy.¹⁵⁻¹⁷ There are two
14 epidemiological studies showing opposite effects of malaria on atopy.^{20,36}
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18 19 **What this study adds**

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21 Using a longitudinal malaria study birth cohort, we identified an association of asthma and
22 atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the
23 increase in risk of malaria associated with these allergic conditions occurred only after the
24 peak clinical incidence of disease in the population, suggesting that they delay the
25 development of clinical immunity to malaria.
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39 children and parents or guardians who participated in the cross-sectional survey '*Pallergo*'.
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3 access to all the data in the study and had final responsibility for the decision to submit for
4 publication.
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6
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9 OMP, AS and RP contributed to the analysis and interpretation of the data. MH, CL, HB, BG,
10 SB, FDS, AF, AT, LB, OMP, SM, AS and RP critically reviewed the report and approved its final
11 version for submission. All authors had full access to all of the data in the study and can take
12 responsibility for the integrity of the data and the accuracy of the data analysis. MH and RP
13 are guarantors.
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16 Competing interests: All authors have completed the ICMJE uniform disclosure form at
17 www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and
18 declare: no financial relationships with any organisations that might have an interest in the
19 submitted work in the previous three years; no other relationships or activities that could
20 appear to have influenced the submitted work.
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22
23 Ethical approval: The allergy study was approved by the Senegalese National Ethics
24 committee (2009/N°46). Renewed approval of the longitudinal malaria study was obtained
25 from the same committee (2006/N°969).
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28 Data sharing: The allergy database will be made available on-line. The longitudinal malaria
29 data set will be made available following discussion with the coordinators of the three
30 Institutes that govern the dataset through contact with the corresponding author.
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Table 1 Classification of Asthma, Allergic rhinoconjunctivitis, Atopic dermatitis and overall Atopic status according to ISAAC questionnaire in children aged 0-14 from a malaria birth cohort. N is total number of children examined and n-malaria represents those for whom malaria data were recorded. F is the number of females and M the number of males.

	N (F/M)	%	n-malaria (F/M)
Asthma symptoms			
None	153 (73/80)	87.43	125 (59/66)
Moderate	4 (1/3)	2.29	4 (1/3)
Severe	18 (6/12)	10.29	14 4/10)
Rhinoconjunctivitis symptoms			
None	146 (64/82)	83.43	120 (52/68)
Moderate	11 (8/3)	6.29	9(6/3)
Severe	18 (6/12)	10.29	14 (6/8)
Atopic dermatitis symptoms			
None	159 (75/84)	90.86	128 (60/68)
Moderate	11 (1/10)	6.29	11 (1/10)
Severe	5 (4/1)	2.86	4 (3/1)
Atopic tendency			
Unlikely	119 (56/63)	68.00	97 (46/51)
Probable	16 (8/8)	9.14	14 (6/8)
Highly probable	40 (16/24)	22.86	32 (12/20)

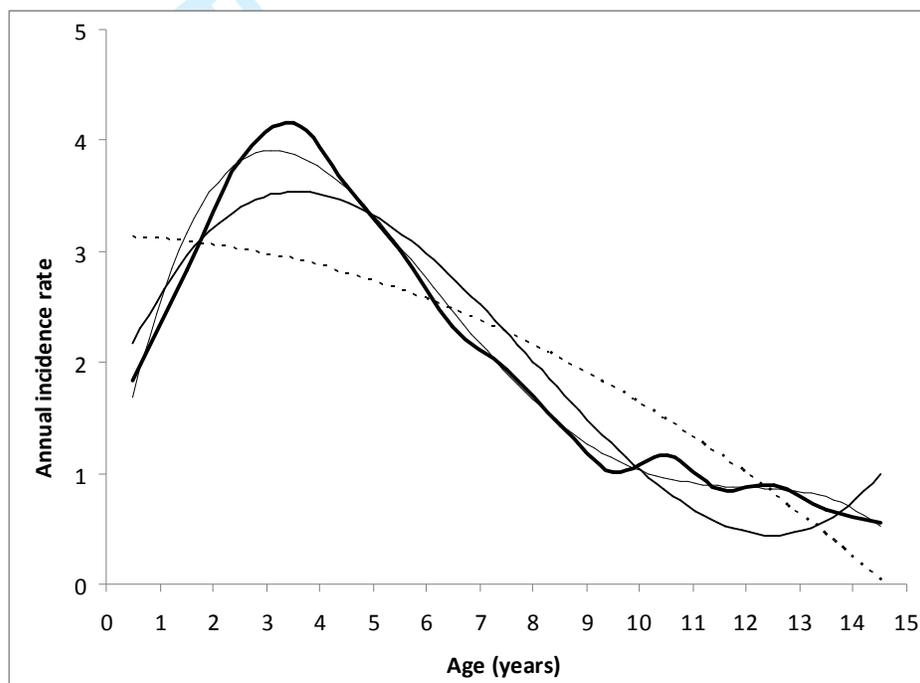
Table 2 Impact of allergy status on risk of *P. falciparum* clinical episodes. Shown are the *P* values and adjusted Odds Ratios with 95% confidence intervals calculated from the mixed model analyses. Values for the covariables Age (≥ 3.5 years of age compared to < 3.5 years of age), Trimestrial incidence of *P. falciparum* clinical episodes and HbAS (beta-globin sickle cell trait; AS compared to AA) are those from the Asthma model analysis. For clarity significant co-variables are shown in bold.

	Age groups < 3.5 years $>$	ORa	95% Confidence Intervals		<i>P</i> value
			Lower	Upper	
Atopy	Both	1.65	1.20	2.26	2.0×10^{-3}
	< 3.5	1.38	0.92	2.08	0.124
	≥ 3.5	2.02	1.39	2.93	2.1×10^{-4}
Asthma	Both	2.12	1.46	3.08	8.0×10^{-5}
	< 3.5	1.50	0.90	2.50	0.122
	≥ 3.5	2.33	1.50	3.61	1.5×10^{-4}
Atopic dermatitis	Both	1.05	0.65	1.70	0.842
	< 3.5	0.84	0.49	1.46	0.539
	≥ 3.5	3.15	1.56	6.33	1.3×10^{-3}
Rhinoconjunctivitis	Both	0.96	0.65	1.41	0.818
	< 3.5	1.05	0.64	1.72	0.853
	≥ 3.5	0.95	0.60	1.52	0.834
Age ≥ 3.5		0.48	0.40	0.57	2.7×10^{-15}
Trimestrial incidence		1.01	1.00	1.01	1.8×10^{-6}
HbAS		0.24	0.12	0.47	3.7×10^{-5}

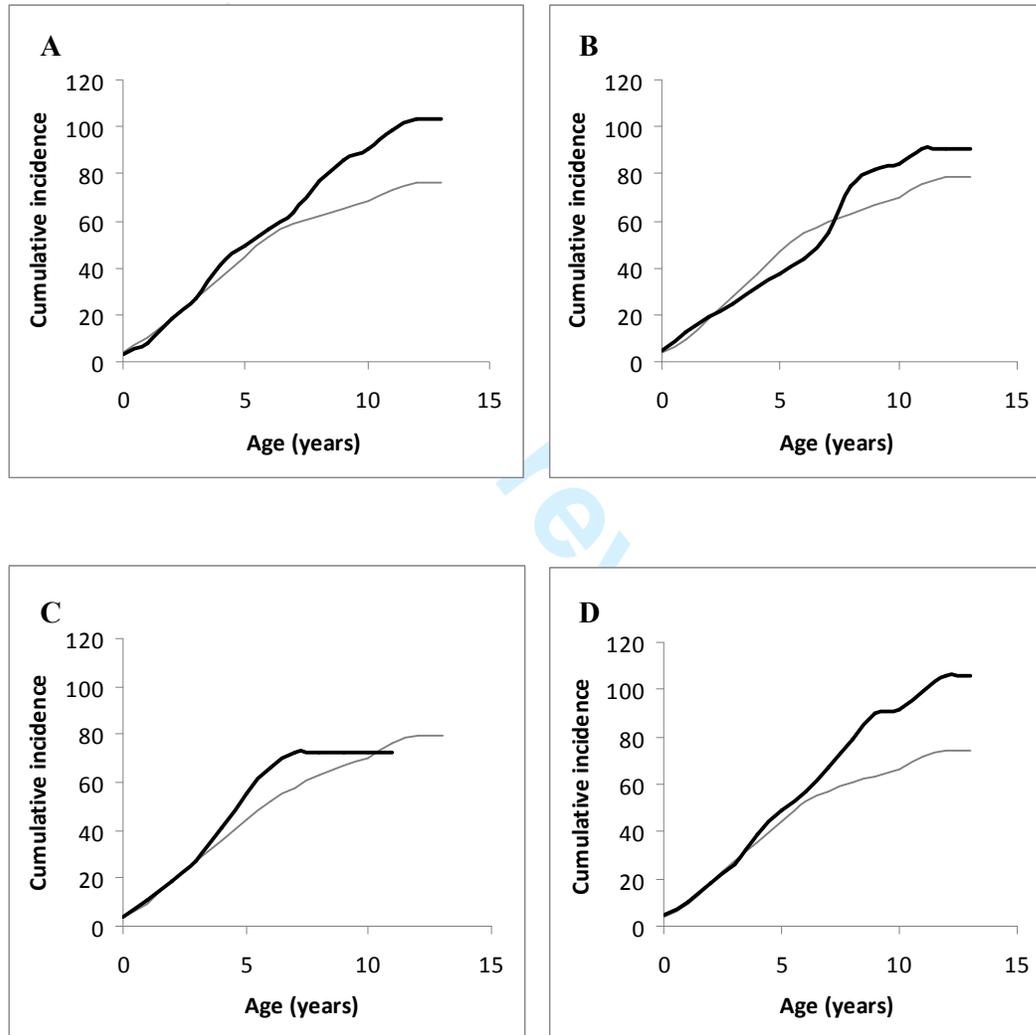
Table 3 Impact of allergy status on the maximum *P. falciparum* parasite density during a clinical malaria episode. Shown are the back-transformed mean parasite densities per microlitre and standard errors (SEM) estimated from the GLMM analyses after taking into account the other co-variables. Significantly different effects are shown in bold for clarity.

Allergic condition	Age groups	Allergic status (No/Yes)	Mean parasite density	SEM	<i>P</i> value	
Atopy	Both	N	76.3	13.8		
		Y	131.0	36.4	0.0158	
	<3.5	N	114.3	23.7		
		Y	171.1	56.0	0.148	
		≥3.5	N	48.4	9.8	
			Y	114.8	37.1	9.5x10⁻⁴
Asthma	Both	N	78.1	14.4		
		Y	148.5	44.3	3.8 x10⁻³	
	<3.5	N	114.8	24.3		
		Y	171.9	74.5	0.167	
		≥3.5	N	51.3	9.7	
			Y	105.3	41.0	6.2 x10⁻³
Atopic dermatitis	Both	N	82.6	15.0		
		Y	93.9	38.9	0.605	
	<3.5	N	122.6	25.5		
		Y	133.9	63.5	0.425	
		≥3.5	N	52.3	11.0	
			Y	135.4	70.7	0.014
Rhinoconjunctivitis	Both	N	81.5	14.8		
		Y	111.4	39.0	0.570	
	<3.5	N	118.8	25.1		
		Y	166.3	69.9	0.537	
		≥3.5	N	54.6	11.3	
			Y	80.9	33.7	0.327

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3 **Figure 1** Annual incidence rate of clinical *P. falciparum* episodes per 100 children (bold
4 line). In order to overcome the fluctuations of the annual incidence rate, we fit second (dotted
5 line), third (dashed line) and fourth (solid line) degree polynomial trend lines to the data (bold
6 line). The corresponding R-squared values are 0.70, 0.91 and 0.99 respectively indicating an
7 accurate fit for third and fourth order polynomials. The inflexion on these two trend lines
8 indicates the onset of acquisition of clinical immunity at approximately 3 to 4 years of age.
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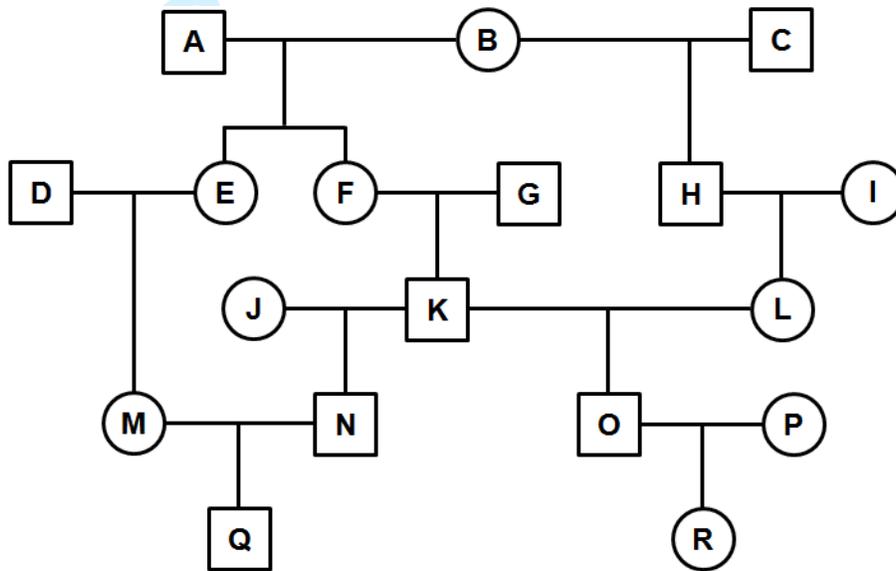
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3 **Figure 2** Mean cumulative number of *P. falciparum* clinical episodes with age for the (A)
4 Asthma, (B) Rhinoconjunctivitis and (C) Atopic dermatitis classes and overall Atopy class
5 (D) (bold lines) compared to individuals without symptoms of each respective allergy type
6 (thin lines). In all cases moderate and severe classes are combined and compared to
7 individuals without allergy symptoms. Note there are no children older than 11 years of age
8 with Atopic dermatitis.
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Pedigree-based genetic relatedness

The Genetic covariance between two individuals can be computed using the pedigree information. For individuals A and B, a given pair in a pedigree, the genetic covariance is computed as $r(A,B) = 2 \times \text{coancestry}(A,B)$ where the *coancestry* between A and B is calculated referring to the method presented by Falconer and Mackay in 1996 (Falconer and Mackay 1996): $\text{coancestry}(A,B) = \sum_p (1/2)^{n(p)} \times (1 + I_{\text{Common Ancestor}})$ where p is the number of paths in the pedigree linking A and B, $n(p)$ the number of individuals (including A and B) for each path p and I_X is the *inbreeding* coefficient of X also equal to the *coancestry* between the two parents of X, I_X is set to 0 if X is a founder.

Illustration: Consider, as an example, the pedigree below containing 18 individuals named {A, B, ..., R} for the calculation of genetic covariance's.



Pedigree structure.

The genetic relatedness between individuals N and O is equal to 0.266. This value is calculated as followed:

The number of paths linking N and O from the pedigree structure above is $p = 2$.

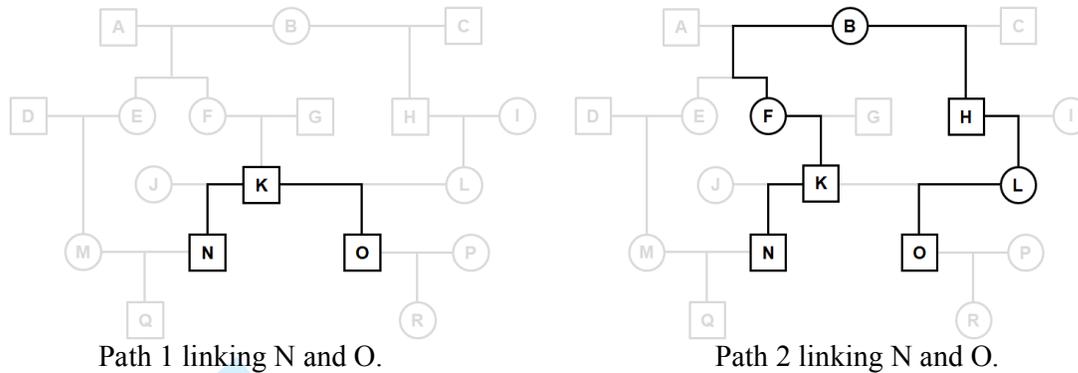
As illustrated below:

- **Path 1** contains $n(1) = 3$ individuals {N, K, O} with K as the common ancestor. Inbreeding coefficient of K, I_K , is the *coancestry* between the two parents of K (F and G) and is null because F and G are not genetically linked.
- **Path 2** contains $n(2) = 7$ individuals {N, K, F, B, H, L, O} with B as the common ancestor. Inbreeding coefficient of B, I_B , is null because B is a founder.

Therefore, genetic relatedness between individuals N and O is:

$$= 2 \times (0.5^{n(1)} \times (1 + I_K) + 0.5^{n(2)} \times (1 + I_B))$$

$$= 2 \times (0.5^3 \times (1 + 0) + 0.5^7 \times (1 + 0)) = 0.266$$



Defining an equivalent model design where individual effects are independent using the genetic relatedness matrix:

Let us rename $Y^* = l(\mu)$. Y^* can be considered as a linearization of the phenotype through the link function l . The expected mean of Y^* and the variance of Y^* are:

- (i) $E(Y^*) = E(X\beta + Z\gamma + \varepsilon)$
 $= E(X\beta) + E(Z\gamma) + E(\varepsilon) = X \times E(\beta) + Z \times E(\gamma) + E(\varepsilon)$
 $= X\beta$ (asymptotically).
- (ii) $\text{Var}(Y^*) = \text{Var}(X\beta + Z\gamma + \varepsilon)$
 $= \text{Var}(Z\gamma + \varepsilon)$ (as $X\beta$ is the fixed part, thus has variance equal to 0)
 $= \text{Var}(Z\gamma) + \text{Var}(\varepsilon)$ (as γ and ε are independent)
 $= Z \times \text{Var}(\gamma) \times Z^T + \text{Var}(\varepsilon)$ (Z^T is the transpose of Z)
 $= Z(A\sigma_g^2)Z^T + I\sigma_r^2$
 $= ZAZ^T\sigma_g^2 + I\sigma_r^2$

If individuals were independent, i.e. $A = I_N$, variance of Y^* could be expressed as $ZZ^T\sigma_g^2 + I\sigma_r^2$. However, using linear algebra theory by the method “Cholesky decomposition of a matrix”, we can show that there is an equivalent expression of the variance of Y^* corresponding to the modeling of data from independent individuals, having γ^* as an equivalent vector of random effects and Z^* an equivalent design matrix relating γ^* to Y^* so that:

$\text{Var}(Y^*) = Z^*(I\sigma_g^2)Z^{*T} + I\sigma_r^2$. $I\sigma_g^2$ is then the covariance matrix of the equivalent independent random individual effects γ^* .

Theorem: Cholesky decomposition of a matrix

If A is a symmetric positive-definite matrix, there is a triangular matrix L so that A can be written as $A = LL^T$. L can be seen as the “square root” of the matrix A .

Note that the genetic relatedness matrix A computed using the pedigree information (Falconer and Mackay 1996) is a positive-definite matrix, unless identical twins are in the pedigree in which case it would be positive semi-definite.

Equivalent model with independent random effects: We set $A = LL^T$ then:

$$\begin{aligned} \text{Var}(Y^*) &= Z(A\sigma_g^2)Z^T + I\sigma_r^2 \\ &= Z(LL^T\sigma_g^2)Z^T + I\sigma_r^2 \end{aligned}$$

$$\begin{aligned}
 &= ZLL^T Z^T \sigma_g^2 + I\sigma_r^2 \\
 &= (ZL)(ZL)^T \sigma_g^2 + I\sigma_r^2 \\
 &= (Z^*)(Z^*)^T \sigma_g^2 + I\sigma_r^2 \quad (\text{where we set } Z^* = ZL)
 \end{aligned}$$

Then, if we define $\gamma^* = L^{-1}\gamma$, we can rewrite the model as:

$$Y^* = X\beta + Z^*\gamma^* + \varepsilon \quad (\text{because } Z\gamma = Z(LL^{-1})\gamma = (ZL)(L^{-1}\gamma) = Z^*\gamma^*),$$

and the γ_i^* are independent, in other terms $\text{Var}(\gamma^*) = I\sigma_g^2$, as demonstrated below:

We assumed that $\gamma \sim N(0, A\sigma_g^2)$. Then $\gamma^* = L^{-1}\gamma$ is also distributed as a multivariate Normal with mean $E(\gamma^*) = L^{-1}E(\gamma) = L^{-1} \times 0 = 0$ and variance:

$$\begin{aligned}
 \text{Var}(\gamma^*) &= (L^{-1}) \times \text{Var}(\gamma) \times (L^{-1})^T \\
 &= (L^{-1}) \times A\sigma_g^2 \times (L^{-1})^T = (L^{-1})LL^T(L^{-1})^T \sigma_g^2 \\
 &= (L^{-1}L)(L^{-1}L)^T \sigma_g^2 \\
 &= I\sigma_g^2
 \end{aligned}$$

The random effects are now independent and then the classical mixed model assuming independence between levels (here individuals) is applied, and the estimate of fixed effects obtained are fine, i.e. corrected for genetic relationships.

References

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Supplementary Tables

Table S1 Number of person-trimesters contributed by number of children by age class and the number who had severe/moderate allergy symptoms, for whom malaria data were also available. AS – Asthma, AD – Atopic dermatitis, RC – Rhinoconjunctivitis. Shown also are the numbers of these individuals suffering from two or all three allergy conditions.

Age group	N° person-trimesters	N° people	AS	AD	RC	AS+AD	AS+RC	AD+RC	AS+AD+RC
]1	7	6	1	2	2	0	1	0	0
]2	21	9	0	1	3	0	0	0	0
]3	48	11	1	1	2	0	0	1	0
]4	119	12	1	2	3	0	0	1	0
]5	102	11	3	4	3	2	1	2	1
]6	125	11	1	1	0	0	0	0	0
]7	303	11	1	2	1	1	0	0	0
]8	340	12	1	1	1	1	0	0	0
]9	362	10	2	0	1	0	1	0	0
]10	610	17	1	0	3	0	0	0	0
]11	77	4	2	1	0	0	0	0	0
]12	484	16	3	0	3	0	1	0	0
]13	390	10	1	0	0	0	0	0	0
]14	105	3	0	0	1	0	0	0	0
Total	3093	143	18	15	23	4	4	4	1

Table S2 Summary of total number of person-trimesters with non-malaria and symptomatic *P. falciparum* clinical presentations and total number of non-malaria episodes according to age class. Given are the number of people contributing to each type of presentation.

	Age group (years)	
	<3·5	≥3·5
Total person-trimesters	1283	1810
People	126	113
Total <i>P. falciparum</i> symptomatic trimesters	963	1102
People	114	108
Total non-malaria episodes	754	1114
People	123	109

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Table S3 Effect of changing age threshold on impact of allergy on the risk of clinical malaria and concomitant parasite density. Given are Odds Ratio with 95% confidence intervals, for clinical malaria episodes and the beta coefficient and standard error for parasite density. Corresponding P values are also given. Values are from the nested GLMM analyses.

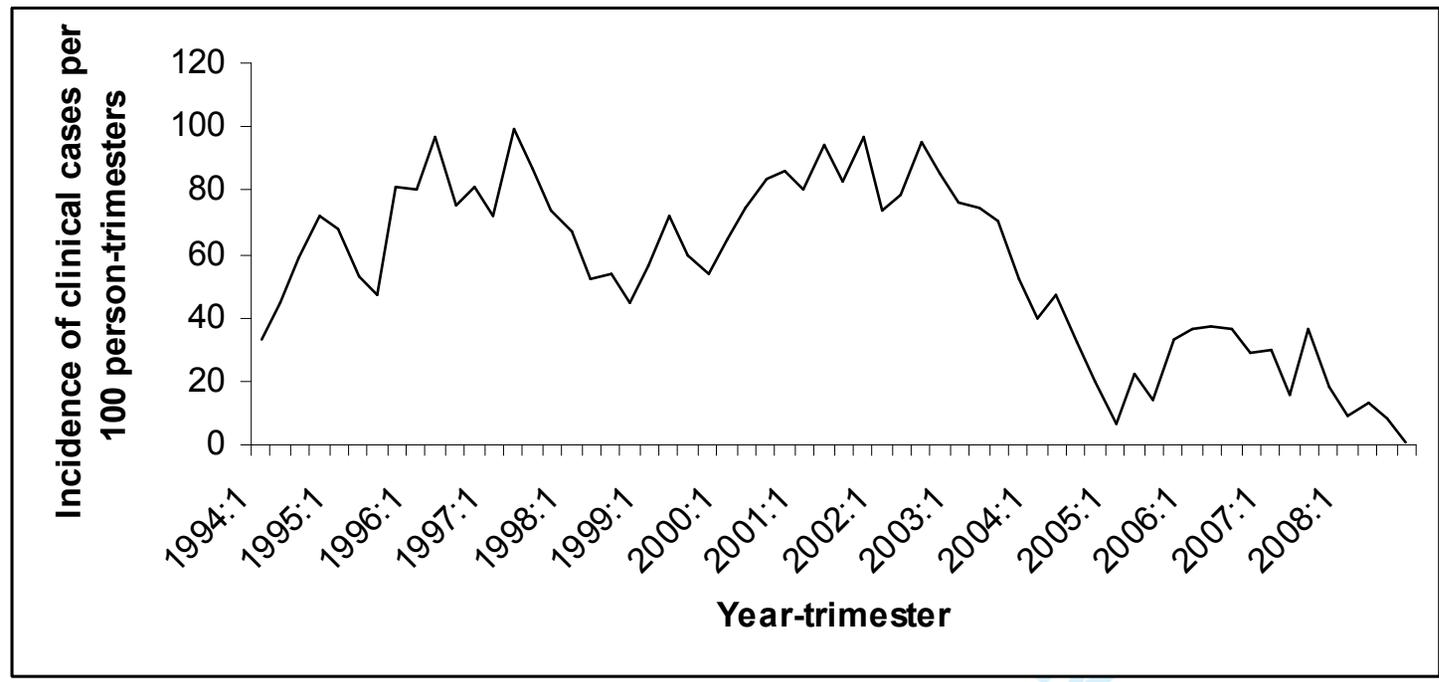
A. Malaria episodes							B. Parasite density					
Age cut-off (years)	OR	95% CI	P value	OR	95% CI	P value	Age cut-off	beta coeff (se)	P value	beta coeff (se)	P value	
	above threshold			below threshold				above threshold		below threshold		
Atopy												
1.5	1.80	1.25-2.59	1.7x10 ⁻³	1.57	0.85-2.89	0.15	1.5	0.70 (0.27)	9.2x10 ⁻³	0.54 (0.35)	0.12	
2.5	2.00	1.39-2.88	2.0x10 ⁻⁴	1.23	0.76-1.99	0.40	2.5	0.79 (0.26)	2.6x10 ⁻³	0.35 (0.29)	0.23	
3.5	2.02	1.39-2.93	2.1x10 ⁻⁴	1.38	0.92-2.08	0.12	3.5	0.85 (0.26)	9.5x10 ⁻⁴	0.37 (0.26)	0.15	
4.5	2.10	1.42-3.10	1.6x10 ⁻⁴	1.41	0.98-2.04	0.063	4.5	0.87 (0.25)	6.9x10 ⁻⁴	0.40 (0.23)	0.09	
5.5	1.64	1.07-2.52	0.02	1.67	1.17-2.37	0.004	5.5	0.73 (0.27)	7.4x10 ⁻³	0.48 (0.22)	3.4x10 ⁻³	
Asthma												
1.5	1.98	1.29-3.03	1.8x10 ⁻³	1.46	0.69-3.19	0.34	1.5	0.66 (0.31)	0.03	0.30 (0.44)	0.48	
2.5	2.30	1.49-3.55	1.6x10 ⁻⁴	1.15	0.63-2.09	0.65	2.5	0.78 (0.30)	0.01	0.26 (0.36)	0.48	
3.5	2.33	1.50-3.61	1.5x10 ⁻⁴	1.50	0.90-2.50	0.12	3.5	0.82 (0.30)	6.2x10 ⁻³	0.43 (0.31)	0.17	
4.5	2.30	1.48-3.59	2.4x10 ⁻⁴	1.76	1.11-2.80	0.017	4.5	0.81 (0.29)	5.8x10 ⁻³	0.56 (0.28)	0.049	
5.5	1.98	1.22-3.22	0.006	2.06	1.33-3.18	0.0011	5.5	0.72 (0.31)	0.02	0.62 (0.27)	0.02	
Atopic Dermatitis												
1.5	2.05	1.18-3.56	0.01	0.91	0.42-1.97	0.80	1.5	0.80 (0.37)	0.03	0.72 (0.46)	0.12	
2.5	2.49	1.36-4.57	3.1x10 ⁻³	0.82	0.44-1.53	0.53	2.5	0.77 (0.38)	0.044	0.52 (0.39)	0.19	
3.5	3.15	1.56-6.33	1.3x10 ⁻³	0.84	0.49-1.46	0.54	3.5	0.99 (0.40)	0.014	0.28 (0.35)	0.42	
4.5	3.79	1.61-8.92	2.3x10 ⁻³	0.94	0.57-1.57	0.82	4.5	0.98 (0.47)	0.036	0.29 (0.32)	0.37	
5.5	1.33	0.47-3.77	0.59	1.19	0.73-1.96	0.49	5.5	0.26 (0.61)	0.67	0.38 (0.31)	0.22	
Rhinoconjunctivitis												
1.5	1.04	0.66-1.62	0.88	1.01	0.51-2.01	0.98	1.5	0.36 (0.32)	0.27	0.18 (0.41)	0.66	
2.5	1.01	0.64-1.61	0.96	0.96	0.55-1.68	0.89	2.5	0.28 (0.33)	0.40	0.25 (0.35)	0.48	
3.5	0.95	0.60-1.52	0.83	1.05	0.64-1.72	0.85	3.5	0.31 (0.32)	0.33	0.19 (0.31)	0.54	
4.5	0.87	0.54-1.42	0.59	1.06	0.68-1.66	0.79	4.5	0.20 (0.32)	0.53	0.22 (0.28)	0.44	
5.5	0.81	0.48-1.36	0.43	1.07	0.70-1.64	0.74	5.5	0.10 (0.33)	0.75	0.23 (0.27)	0.39	

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4 **Table S4 Frequency of non-malaria episodes (number of days of presence divided by number of non-malaria episodes) according to allergic status**
5 **and age group.** The *P* value is that from the GLMM analyses of the effect of allergic status by age group on the number of non-malaria episodes per person-
6 trimester.
7

Allergic condition	Allergic status (No/Yes)	Age group (years)		<i>P</i> value
		<3·5	>3·5	
Atopy	N	78·2	85·9	0·105
	Y	87·2	102·6	
Asthma	N	79·6	87·3	0·319
	Y	82·5	100·2	
Atopic dermatitis	N	80·9	88·2	0·323
	Y	73·4	101·9	
Rhinoconjunctivitis	N	77·9	88·3	0·167
	Y	94·9	91·8	

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Figure S1. Incidence of clinical cases per 100 person-trimesters in children under 15 years of age.



Only

Figure S2. Cumulative incidence of clinical cases according to allergy class predicted by the statistical model.

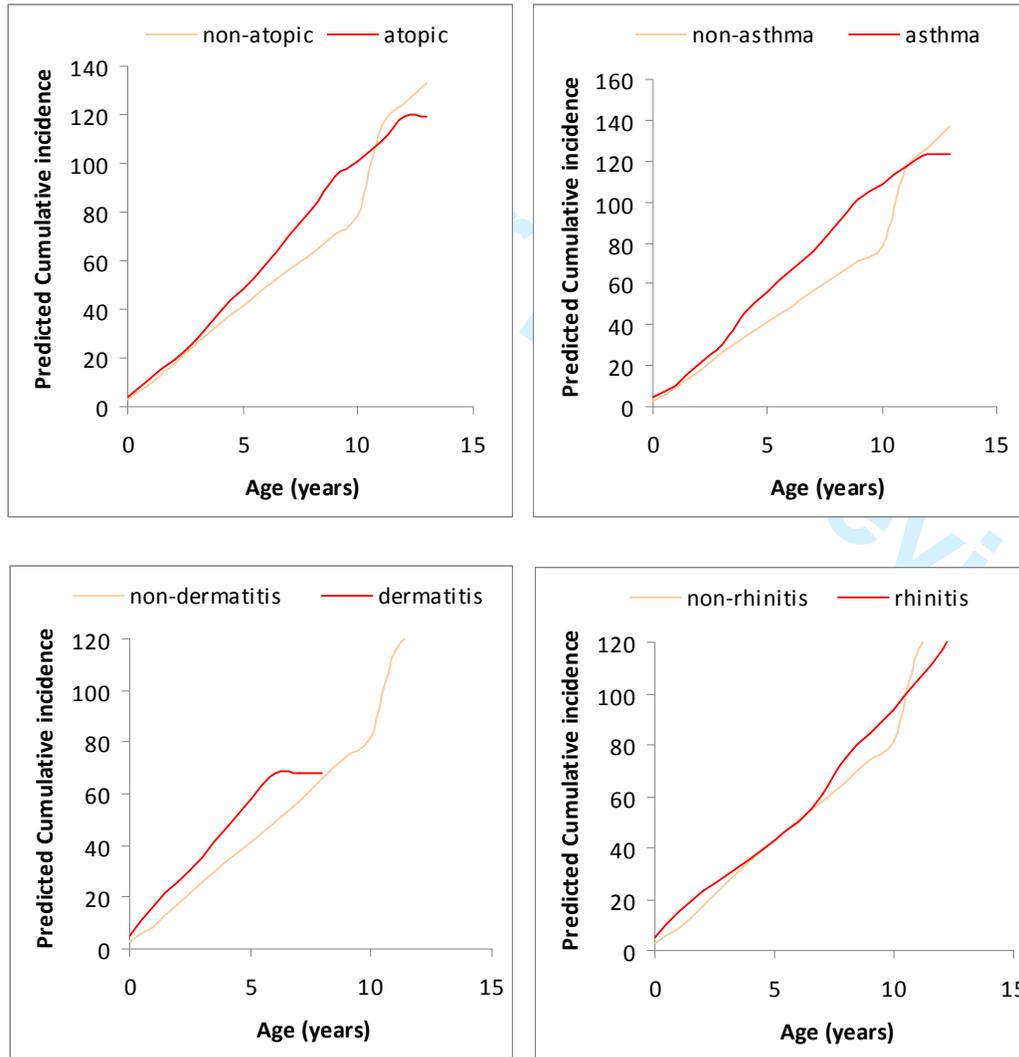
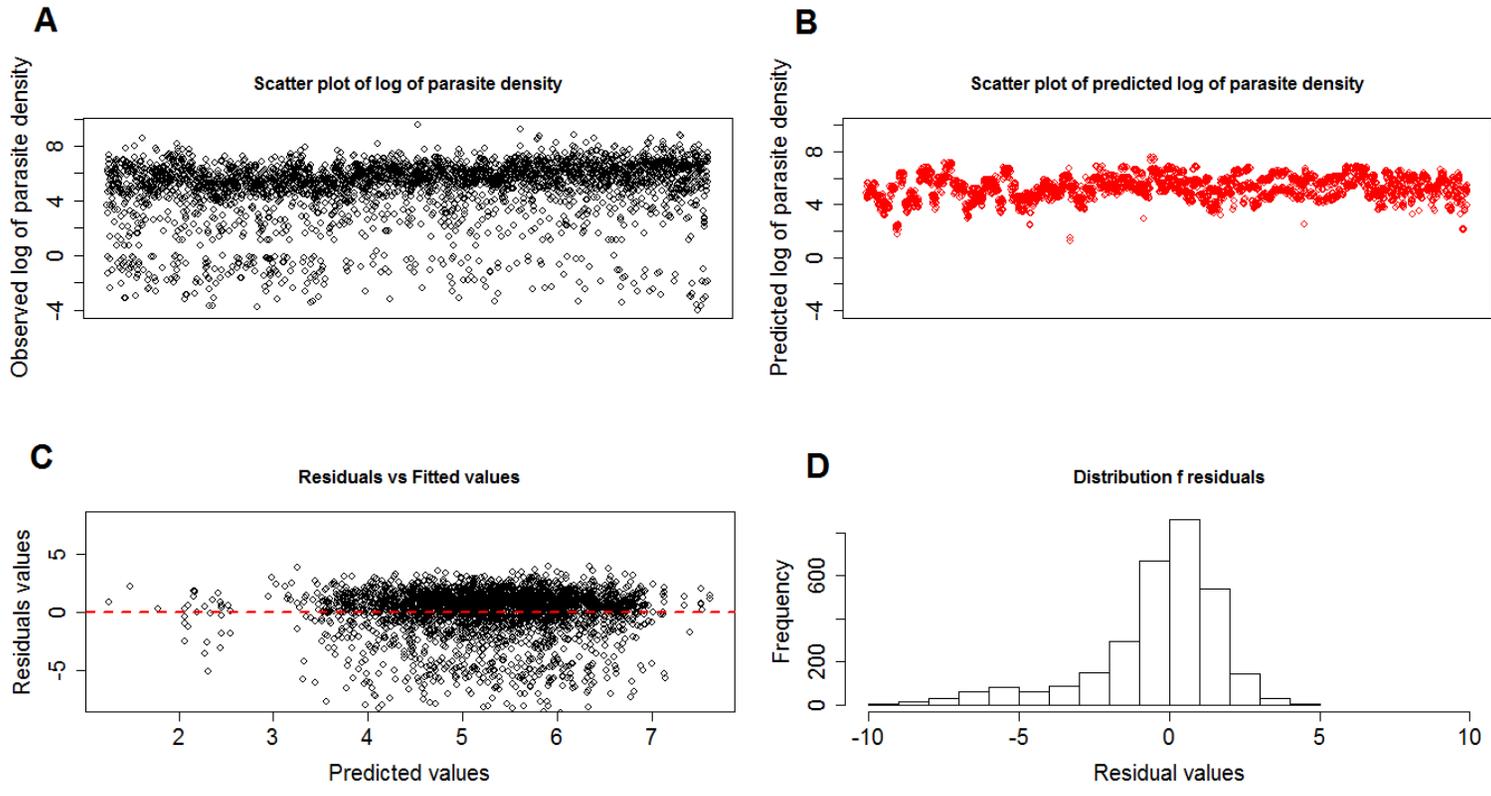


Figure S3. Graphical control model for parasite density

These figures provide a graphical checking of model goodness of fit. Figure A is the scatter plot of the natural logarithm of the observed parasite density and is compared to Figure B, which is the scatter plot of the natural logarithm of the predicted parasite density by the model; on both figures A and B the y-axes give the values for the log of the parasite density. Figure C shows the distribution of the residuals with the predicted values and Figure D is the histogram of the residuals; both figures C and D show the residuals normally distributed around zero.



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7 **Asthma and atopic dermatitis are associated with increased risk of clinical *Plasmodium***
8 ***falciparum* malaria**
9

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Article summary

Article focus

- Genetic studies suggest a link between susceptibility to allergy and malaria in Africa
- We hypothesize that atopy increases susceptibility to malaria

Key messages

- Results demonstrate an association between asthma, atopic dermatitis and susceptibility to clinical *P. falciparum* episodes.
- Genetic pre-disposition to asthma or atopic dermatitis impairs the acquisition of clinical immunity to malaria.
- Administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Strengths and limitations

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association.

Abstract

Objectives: To assess the impact of atopy and allergy on the risk of clinical malaria.

Design: A clinical and immunological allergy cross-sectional survey in a birth cohort of 175 children from 1 month to 14 years of age followed for up to 15 years in a longitudinal open cohort study of malaria in Senegal. Malaria incidence data were available for 143 of these children (aged 4 months to 14 years of age) for up to 15 years. Mixed model regression analysis was used to determine the impact of allergy status on malaria incidence, adjusting for age, sickle cell trait and force of infection.

Main outcome measures: Asthma, allergic rhino-conjunctivitis and atopic dermatitis status, the number of clinical *Plasmodium falciparum* malaria episodes since birth and associated parasite density.

Results: Twelve percent of the children were classified as asthmatic and ten percent as having atopic dermatitis. These groups had respectively a two-fold (OR 2.12 95% confidence intervals 1.46% to 3.08%; $P=8 \times 10^{-5}$) and three-fold (OR 3.15, 1.56% to 6.33%; $P=1.3 \times 10^{-3}$) increase in the risk of clinical *P. falciparum* malaria once older than the age of peak incidence of clinical malaria (3 to 4.5 years of age). They also presented with higher *P. falciparum* parasite densities (Asthma: mean 105.3 parasites/ μ L \pm SE 41.0 vs. 51.3 \pm 9.7; $P=6.2 \times 10^{-3}$; Atopic dermatitis: 135.4 \pm 70.7 vs. 52.3 \pm 11.0; $P=0.014$). There was no effect of allergy on the number of non-malaria clinical presentations. Individuals with allergic rhinoconjunctivitis did not have an increased risk of clinical malaria nor any difference in parasite densities.

Conclusion: These results demonstrate that asthma and atopic dermatitis delay the development of clinical immunity to *P. falciparum*. Despite the encouraging decrease in malaria incidence rates in Africa, a significant concern is the extent to which the increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines, administration to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Introduction

The World Allergy Organization estimates that 40% of the world's population is concerned by allergic diseases.¹ In developing countries where *Plasmodium falciparum* malaria is endemic, prevalence of allergy is significantly lower, but is on the increase.² Orientation of the immune response towards a Th1 profile is crucial for immunity to intracellular pathogens,³ whereas orientation towards a Th2 profile drives immunity to extracellular pathogens and antigens resulting in class switching giving rise to IgE-producing B cells.⁴ An important role of the Th1/Th2 balance in the development of clinical malaria following infection by *P. falciparum* has been suggested by numerous studies.⁵⁻⁷ It has been suggested that the Th2 bias induced by *P. falciparum* may exacerbate allergy.⁸ Likewise, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*. However, the interplay between infectious agents and allergy is unclear. On the one hand, for example, severe respiratory syncytial virus infection in infants increased the risk of allergic rhinoconjunctivitis and allergic asthma.^{9,10} On the other hand, measles,¹¹ hepatitis A¹² and tuberculosis¹³ seemingly reduce atopy. Although, an atopic condition can increase incidence of disease, such as the case for the skin commensal *Staphylococcus aureus* in patients with atopic dermatitis,¹⁴ an atopic tendency *per se* does not generally lead to increased illness from infectious agents.

Plasmodium falciparum specific IgE is elevated in malaria patients and has been proposed to play a pathogenic role in severe malaria.³ T helper type 2 (Th2) cell bias induced by *P. falciparum* may exacerbate allergy.⁴ Genome wide studies have identified chromosomal regions linked to clinical malaria, all of which overlap with those previously identified to be involved in atopic dermatitis, asthma, atopy and IgE levels^{5-7, 15-17} suggesting that common mechanisms may be involved in both pathologies.¹⁸ Chromosomal region 5q31 that has been repeatedly shown to be associated with control of parasite density and contains a cluster of cytokines, among which IL12B has been previously associated with psoriasis.¹⁹ The other regions, 13q13-q22, 5p15-p13 and 12q21-q23, contain genes involved in innate immunity, notably the interleukin 7 receptor, and several involved in tumour necrosis factor synthesis [C1q and tumour necrosis factor related protein 3 (C1QTNF3)] and a gene involved in the complement system (C9).¹⁸

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Several additional lines of evidence support the concept that susceptibility to malaria and atopy may be related to similar immunological defects. In Ethiopia, a history of malaria was associated with atopy.⁹²⁰ A mouse model for human atopic disease was found to be very susceptible to murine malaria and a major locus for atopic disease mapped close to the region controlling parasite density.²¹⁴⁰ This region contains several candidate genes that have effects on T-cell function.²¹

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Moreover, a direct effect of histamine in the malaria pathogenesis has been found using genetic and pharmacological approaches⁴⁴²² and increased levels of histamine are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.^{12,213,24}

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To test the hypothesis that allergy impacts upon clinical *P. falciparum* malaria, we performed a clinical allergy cross-sectional study in the family-based longitudinal cohort from Senegal previously used for the genome linkage study⁸¹⁸ and analysed the impact of asthma, atopic dermatitis, allergic rhinoconjunctivitis on the incidence of clinical *P. falciparum* episodes and the maximum parasite density during each episode.

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Methods

Population and outcome data

The malaria research program conducted in Dielmo village in Senegal has been ongoing since 1990 as described elsewhere.²⁴⁵⁴ In brief, between 1990 and 2008, a longitudinal study involving the inhabitants of the village of Dielmo, Senegal, was carried out to identify all episodes of fever. The study design included daily medical surveillance with systematic blood testing of individuals with fever and examination of 200 oil-immersion fields on a thick blood film for malaria parasites (about 0.5 µL of blood). Each individual was given a unique identification code and details of family ties, occupation, and precise place of residence were recorded on detailed maps of each household with the location of each bedroom. All households were visited daily, absenteeism recorded, and the presence of fever or other symptoms assessed. We systematically recorded body temperature at home three times a week (every second day) in children younger than 5 years, and in older children and adults in cases of suspected fever or fever-related symptoms. In cases of fever or other symptoms,

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7 blood testing was done at the dispensary by finger prick, and we provided detailed medical
8 examination and specific treatment. Parasitologically confirmed clinical malaria episodes
9 were treated according to national guidelines. From 1990 to 2008, four different drug
10 regimens were implemented: Quinine from 1990 to 1994, Chloroquine from 1995 to 2003,
11 Fansidar (sulfadoxine-pyrimethamine) from 2004 to mid-2006 and Artemisinin-based
12 combination therapy (ACT; Amodiaquine- sulfadoxine-pyrimethamine) from mid-2006 to
13 2008.

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18 Parasite positivity was established as follows. Thick blood films were prepared and stained
19 by 3% Giemsa stain. Blood films were examined under an oil immersion objective at x1000
20 magnification by the trained laboratory technicians and 200 thick film fields were examined
21 to count the number of asexual and gametocyte parasite stages. Asexual parasite densities
22 (per μ L) were calculated by establishing the ratio of parasites to white blood cells and then
23 multiplying the parasite count by 8,000, the average white blood cell count per μ L of blood.

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28 Malaria transmission in Dielmo is intense and perennial. We conducted a cross-sectional
29 survey to estimate the prevalence of symptoms related to allergic diseases among 175
30 children ~~aged from 1 month to 14 years old below the age of 15 years~~ who were born during
31 the malaria research program.

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34 Both the longitudinal and cross-sectional surveys were approved by the Ministry of Health of
35 Senegal. Informed consent of the volunteers is renewed every year. More specifically for the
36 cross-sectional survey, after informing about the procedures and the purpose of the study,
37 written informed consent was obtained from parents or guardians of children either by
38 signature or by thumbprint on a voluntary consent form written in both French and Wolof,
39 the main local language. Consent was obtained in the presence of the school director, an
40 independent witness.

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45 The family structure (pedigree) was available after a demographic census performed for
46 every volunteer at his adhesion in the project. A verbal interview of mothers or key
47 representatives of the household was used to obtain information on genetic relationships
48 between studied individuals, their children, their parents, and to identify genetic links
49 among the population. The total pedigree comprised 828 individuals, including absent or
50 dead relatives, composed of ten independent families that can be sub-divided into 206
51 nuclear families (father – mother couples with at least one child) with an average of 3.6

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children each. Genetically related nuclear families occur because of multiple marriages and marriages among related individuals. Previous typing with microsatellites has enabled the construction of a pedigree based on Identity-by-Descent using MERLIN.^{18,26} The mean coefficient of inbreeding is 0.0008. Newborns since this original genetic analysis were added to the family of the parents in question. The 143 children, with both allergy and malaria data, belonged to 61 nuclear families and comprised 30 singletons, 102 siblings and 11 half-sibs (yielding 55 half-sib pairs). The mean genetic relatedness (by pedigree) of the 143 children is 0.0114 (range: 0.0013 to 0.022).

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P. falciparum clinical episodes

P. falciparum malaria clinical episode phenotypes analysed were: (i) clinical *P. falciparum* infections treated with anti-malarial therapy and (ii) the highest parasite density during the *P. falciparum* clinical episode. A clinical *P. falciparum* episode was defined as a clinical presentation with fever (axillary temperature $\geq 37.5^{\circ}\text{C}$) and/or other clinical signs suggestive of malaria associated with a thick blood smear positive for *P. falciparum* and that was treated with anti-malarial therapy. The parasite density was measured from 200 microscope fields on a thick blood smear (0.25 μL blood). Repeated clinical malaria presentations within 15 consecutive days were not considered to be independent and were excluded from the analyses, unless there was a negative thick blood smear between two clinical presentations. We also excluded observations in any trimester for which the individual was not present for at least one third of the time.

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We calculated the quarterly incidence rate of clinical *P. falciparum* episodes in children below the age of 15 years as the ratio of the total number of clinical *P. falciparum* episodes during the trimester divided by the total number of person-trimesters surveyed. Incidence rate is expressed as cases per 100 person-trimesters (see Supplementary Figure S1). This rate was used in the analysis to approximate the force of infection (exposure level) within the targeted population at the time of a given clinical *P. falciparum* episode.

The total number of clinical presentations per trimester that were not attributable to *P. falciparum* was tabulated. Repeated non-malaria presentations within seven consecutive days were not considered to be independent and were excluded.

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Allergic diseases and atopic status

The International Study of Asthma and Allergies in Childhood (ISAAC) diagnostic criteria have been shown to be reproducible, adequate and able to discriminate children with allergic diseases in different areas of the world.² The standardized ISAAC questionnaire originally written in English was translated into French in compliance with ISAAC guidelines¹⁵²⁷, adapting it to the usual local customs following advice from local clinicians and paediatric allergologists ([Acknowledgements and Technical Appendix](#)). The adequacy and reliability of the translated questionnaire had been previously confirmed by a pilot study on 30 randomly selected children in the same community. The questionnaire was completed by specially trained health workers during an oral interview conducted in Wolof with children and their mothers or guardians.

To assess the prevalence of allergic diseases in children, we used the positive and negative predictive values of the ISAAC questionnaire diagnosis criteria developed for subtropical countries.¹⁶²⁸ Each question was scored according to the medical diagnosis of paediatricians and paediatric allergologists. Positive or negative answers were thus graded on the basis of symptom sensitivity, specificity, frequency, location or early onset. For each allergic disease, three categories of symptom severity, *severe*, *moderate*, and *none*, were defined as follows:

Asthma – severe symptoms if the child had “wheezing or whistling in the chest before the age of two years” and “more than three times” or severe enough to “limit his/her speech”; *moderate* symptoms if the child had “wheezing or whistling in the chest before the age of two years” and “in the past 12 months”; and *none* otherwise.

Allergic rhinoconjunctivitis – severe symptoms if the child had “sneezing, runny or stuffy nose in the past 12 months” and “more than five times a year”, and “itchy, watery eyes or tropical endemic limboconjunctivitis (TELC) in the past 12 months”; *moderate* symptoms if the child had “sneezing, runny or stuffy nose in the past 12 months”, and “itchy, watery eyes or TELC in the past 12 months”; and *none* otherwise.

Atopic dermatitis – severe symptoms if the child had “scaly or exudating, crusted and pruritic patches in the past 12 months” and “affecting any of the following characteristic areas: face, around the ears or eyes, folds of armpits or elbows or groin, behind the knees, under the buttocks”, and “onset of symptoms before the age of two years”; *moderate* symptoms if the

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child had “scaly or exudating, crusted and pruritic patches in the past 12 months” and “affecting any of characteristic areas (see above)”, and “onset of symptoms before the age of four years”; and *none* otherwise.

The inter-relationships between variables reflecting the severity of symptoms of the three allergic diseases were used to identify children at high risk of atopy. The *high probability* group was defined by the prevalence of at least one of any *severe* symptoms or two of any *moderate* symptoms. The *probable* group was defined as those with *moderate* symptoms from one of the three allergic diseases and remaining children were classified in the *unlikely* group.

Helminths

Helminthic infections are common in this region and are known to modify the clinical course and outcome of both allergic diseases and malaria.^{29,30} We therefore carried out a helminth survey ~~was carried out~~ for 91 individuals present during the cross-sectional survey. Diagnosis was performed by stool examination by microscope and by the Kato technique to search for the presence of *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*), whipworm (*Trichuris trichiuria*), *Schistosoma mansoni*, and *Strongyloides stercoralis*. Examination for pinworms (*Enterobius vermicularis*) was performed by the anal scotch-test. An anti-helminthic treatment was proposed for all infested individuals.

Immunoglobulin E titres

Specific IgE titres were measured by ELISA as previously described.^{31,47} A panel of allergens of potential pertinence to the three classes of allergy was used: (i) Salivary gland extracts (SGE) of two mosquito species present in the study cohorts, *Aedes aegypti* and *Anopheles gambiae sensu stricto*, and (ii) *P. falciparum* parasite extract were prepared as previously described^{31,47}; (iii) House dust mite spp. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*; (iv) a mix of pollen allergens from five ubiquitous gramineae spp. [Cock’s-foot (*Dactylis glomerata*), Timothy grass (*Phleum pratense*), Sweet Vernal grass (*Anthoxanthum odoratum*), Perennial ryegrass (*Lolium perenne*), Kentucky Bluegrass (*Poa pratensis*)] (all from Stallergenes, France).

Statistical analysis

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Statistical analyses were performed using R version 2.12.0 (The R Foundation for Statistical Computing, Vienna, Austria). To address the effect of allergic status on the risk of clinical *P. falciparum* episodes, we performed Generalized Linear Mixed Models (GLMM) extended to pedigree data using the *pedigreemm* package for R to account for the non-independence of individuals because of family relationships, shared house and for repeated measures from the same individual (Technical Appendix). Correlated individual effects due to familial relationships were taken into account by using the pedigree-based genetic relatedness matrix that contains the genetic covariance among all pairs of individuals in the study cohort and is calculated using the pedigree information.¹⁸³² Shared house and repeated measures from the same individual were modelled as random effects. All random effects were assumed to be normally distributed, and conditional on these random effects, the dependent variable had: (i) a Binomial distribution when the studied phenotype was the occurrence of a clinical *P. falciparum* episode treated with anti-malarial therapy during a trimester, (ii) a Gaussian distribution when the studied phenotype was the logarithm of the maximum parasite density during a given clinical *P. falciparum* episode, and (iii) a Poisson distribution when the studied phenotype was the number of non-malaria episodes per trimester. The effects of allergy disease classes on these dependent variables were modelled as fixed effects. Allergy classes were reduced to two levels, *Severe* or *moderate* vs. *none* for analyses of asthma, atopic dermatitis and allergic rhinoconjunctivitis and *high probability* vs. *probable* and *unlikely* for atopic tendency. Co-variables included sickle cell trait¹⁷³¹, *gender*, number of days present on site during the trimester, trimestrial incidence of *P. falciparum* and age. Age was initially analysed as a continuous covariate. To assess the age-specific effect of allergy, age was categorised into two levels (<3.5 years of age and ≥3.5 years of age, based on the age of peak clinical incidence) and allergy class was nested within age class. The age threshold was varied from 1.5 years to 5.5 years of age and the data re-analysed to assess at which age there was the strongest effect. The association of allergy classes with IgE levels was analysed by box-cox transforming the data and fitting a GLMM with ~~Poisson~~ a normal distribution.

Results

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7 Of the 205 eligible children aged under 15 years involved in the family-based longitudinal
8 study, 175 (85.4 %) participated in the cross-sectional survey to assess the prevalence of
9 related symptoms of allergic diseases. All eligible children present at the time of the survey
10 were included; no explicit refusal to participate was recorded. The study cohort was aged
11 from 1 month to 14 years 11 months. The sex-ratio (male/female) was 0.94.

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14 From 1994 until 2008, 143 of the children participating in the cross-sectional survey were
15 present for at least 31 days in any trimester during the study period generating a total of
16 3,093 person-trimesters of presence (Supplementary Table S1). There were 2,065 treated *P.*
17 *falciparum* clinical episodes (median 11, range 0-47)(Supplementary Table S2). The age peak
18 of incidence of *P. falciparum* episodes occurred at 3 to 4.5 years of age (Figure 1). There
19 were 1,868 non-malaria episodes (median 12, range 0-37) (Supplementary Table S2). These
20 non-malaria clinical presentations were associated with headache (38 %), chills (32 %), cough
21 (13 %), vomiting (11 %) and diarrhoea (6 %).

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24 The prevalence of moderate or severe asthma symptoms was respectively 2.3 % and 10.3 %
25 (Table 1). The prevalence of moderate or severe allergic rhinoconjunctivitis symptoms was
26 respectively 6.3 % and 10.3 %. The prevalence of moderate or severe atopic dermatitis
27 symptoms was respectively 6.3 % and 2.9 %. On the basis of symptom severity, an atopic
28 tendency was estimated to be unlikely for 68.0 %, probable for 9.1 % and highly probable for
29 22.9 % of the 175 children. The frequency of each allergy class in children for whom malaria
30 data were available is shown in Table S1.

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33 The risk of treated clinical *P. falciparum* infections was higher for children with high
34 probability of atopy (OR 1.65, 95% confidence intervals 1.20 to 2.26; P=0.002) (Table 2), after
35 adjusting for age, sickle cell trait and the exposure level. Gender was not found to be
36 significant. Analysing the impact of atopy in children younger and older than the peak age of
37 clinical incidence (3 to 4.5 years old), revealed that atopy doubled-increased the risk of *P.*
38 *falciparum* episodes in children at an age greater than 3.5 years (OR 2.02, 1.39 to 2.93;
39 P=2x10⁻⁴), but not in children of age prior to the peak clinical incidence (OR 1.38, 0.92 to
40 2.08; P=0.124) (Table 2). This increased risk resulted in an ever increasing cumulative
41 number of *P. falciparum* episodes with age beyond that of peak clinical incidence (Figure 2.
42 See supplementary Figure S2 for model predictions for comparison).

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Analysis by allergy category revealed that asthma (severe or moderate) ~~doubled-increases~~ the risk of *P. falciparum* episodes (OR 2.12, 1.46 to 3.08; $P= 8 \times 10^{-5}$) and this again only in children of age greater than 3.5 years old (OR 2.33, 1.50 to 3.61; $P= 1.5 \times 10^{-4}$). Atopic dermatitis ~~tripled-increased~~ the risk of clinical malaria in children older (OR 3.15, 1.56 to 6.33; $P= 1.3 \times 10^{-3}$) but not younger than 3.5 years of age (Table 2). Allergic rhinoconjunctivitis was not associated with increased risk of clinical malaria at any age (Table 2). The impact of atopy, asthma and atopic dermatitis can be clearly seen in the ever-increasing number of cumulative *P. falciparum* episodes beyond the age of the onset of clinical immunity in the population, 3.5 years of age (Figure 2). ~~There is no difference in the number of clinical malaria episodes prior to this age in individuals with or without an allergic condition. Analysis using different age thresholds (from 1.5 to 5.5 years of age) revealed similar OR for thresholds of 2.5, 3.5 and 4.5 years of age. The maximum OR for increased malaria occurred in children older than 4.5 years of age and with atopy or atopic dermatitis, whereas for the asthma group it occurred in children after 3.5 years of age (Supplementary Table S3). There is no difference in the number of clinical malaria episodes prior to this age in individuals with or without an allergic condition.~~

There was no impact of any allergic disease on the number of non-malaria episodes by trimester (~~Supplementary Table S4~~).

The impact of atopy, asthma and atopic dermatitis on the maximum *P. falciparum* parasite density during a given clinical malaria episode mirrored that of the risk of *P. falciparum* episodes. Parasite density was significantly higher for children with allergic disease older than 3.5 years of age (Table 3 ~~and supplementary Figure S3 for residuals of the fitted model~~). Allergic rhinoconjunctivitis had no impact on the parasite density (Table 3). ~~Analysis using different age thresholds yielded the same pattern as seen with the number of clinical episodes (Table S3).~~

Individuals with moderate or severe symptoms of atopic dermatitis had significantly higher specific IgE titres against *Ae. aegypti* ($P=0.004$) and *An. gambiae* SGE ($P<0.001$). There were no detectable specific anti-*P. falciparum* IgE. Individuals with moderate or severe symptoms of allergic rhinoconjunctivitis did not have significantly higher IgE titres against the tested graminiae ($P=0.28$), although titres decreased with age ($P=0.035$). There was also no effect of

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7 asthma on IgE titres against the house dust mite spp. tested (*D. farinae* P=0.60 & *D.*
8 *pteronyssinus* P=0.27).

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10 Only five individuals were infested with helminths (two *Ancylostoma*, one *Strongyloides*, one
11 *Trichuris* and one *Enterobius*).

12 13 14 15 **Discussion**

16 17 **Principal findings**

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19 Establishing the allergic status of children up to the age of 15 years old followed for malaria
20 since birth, revealed an association of asthma and atopic dermatitis with susceptibility to
21 clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with
22 these allergic conditions occurred after the peak clinical incidence of disease in the
23 population, suggesting that they delay the development of clinical immunity to malaria.
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27 **Strengths and weaknesses of the study**

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29 The major strength of this study is the complete knowledge of the number of clinical *P.*
30 *falciparum* malaria episodes each individual has had since birth and the exposure level per
31 trimester over the 15 years covering the birth cohort. No other study has such detailed
32 information for such a length of time. The major weakness of the study is the relatively small
33 sample size, which would have reduced power to detect an association. In addition, although
34 allergy diagnosis for children under 2 years of age is not considered reliable, there were only
35 15 individuals under 2 at the time of the allergy study of the 143 for whom malaria and
36 allergy data were available.
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42 **Meaning of the study**

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44 Under intense malaria transmission, after repeated exposure to the parasite, children
45 develop a clinical immunity⁴⁹³³, whereby they tolerate elevated parasite densities without
46 showing clinical symptoms. In this cohort, the population mean onset of clinical immunity
47 occurred at 3 to 4.5 years of age. Although clinical immunity is accompanied by a reduction
48 in parasite density, effective anti-parasite immunity develops much more slowly²⁰³⁴ with
49 individuals achieving a state of premunition, whereby they maintain low-grade parasite
50 densities in an asymptomatic state.²⁴³⁵ We show here that children with clinically defined
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asthma or atopic dermatitis had a two to three-fold increase in the risk of presenting with *P. falciparum* malaria episodes requiring treatment once passing the age of peak clinical incidence. They also had higher parasite density during clinical episodes, suggesting a reduced ability to control parasite replication. The observed increase in clinical incidence of malaria in patients with asthma or atopic dermatitis is not likely to be the result of increased frailty of such individuals; these individuals did not come more frequently to the clinic with non-malaria symptoms. Our previous genome linkage study identifying chromosomal regions⁸¹⁸ associated with malaria that overlap with those previously shown to be linked to asthma/atopy suggests that there may be a shared genetic basis to these pathologies rather than any causative effect of one on the other. This is consistent with the increased susceptibility to malaria of mouse atopic models.⁴⁰²¹

Comparison with other studies

A previous study found that a history of malaria (yes/no) increased risk of atopic dermatitis in 306 cases compared to 426 controls as characterized using the ISAAC questionnaire⁹²⁰. The only other epidemiological study that has previously examined the link between malaria and atopy²²³⁶ also interpreted the result from the perspective of the impact of malaria on atopy. They examined the re-infection rate with *P. falciparum* over a 5-year period in 91 children that were subsequently classified as atopic or not using skin prick tests (SPT) with house dust mite antigen. Their conclusion was that, as with measles²²¹¹ and tuberculosis²⁴¹³, malaria infection reduces atopy. However, the study lacked previous infection data since birth of the participating individuals and focussed on atopy as determined by SPT against a single allergen. The case-control study of atopic dermatitis risk factors cited above found no overall association between allergen skin sensitization and atopic dermatitis. We also found no evidence of increased IgE titres against house dust mites in the asthmatic or atopic dermatitis groups or against grass pollen in individuals with allergic rhinoconjunctivitis. Such differences likely reflect the different IgE reactivity profiles due to differences in allergen exposure in Africa.³⁷ There was no evidence of anti-parasite IgE in this cohort of children. We previously showed that circulating anti-parasite IgE titres were strongly positively correlated with anti-mosquito saliva IgE, but became undetectable following malaria exposure, potentially being bound to effector cells.³¹ Only mosquito saliva, a known major local

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7 allergen, induced a specific IgE response at significantly higher titres in individuals with
8 atopic dermatitis.

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10 Although the immune effectors of clinical immunity are still poorly defined, there is strong
11 evidence that acquired anti-parasite immunity is IgG-dependent³⁸ and cytophilic
12 immunoglobulins (IgG1 & IgG3), which are capable of eliminating the parasites by
13 opsonisation and/or by Antibody Dependent Cellular Immunity play an important role in
14 premunition.³⁵ The higher parasite density during symptomatic episodes observed in the
15 asthma group suggests impaired development of acquired immunity. Impaired acquisition of
16 immunity to malaria in children with asthma or atopic dermatitis may stem from their
17 imbalanced Th1/Th2 response. Indeed, an atopic state may generate a tendency to develop
18 a Th2 type immune response to *P. falciparum*. Dendritic cells that are oriented to a Th2
19 phenotype are more susceptible to orient the acquired immune response towards a Th2
20 profile.³⁹ Orientation of the immune response towards a Th2 profile by asthma or atopic
21 dermatitis would result in a poor Th1 response (and hence development of protective IgG
22 immunoglobulins), considered to be the dominant arm of the immune response enabling
23 resistance to infectious disease in children.⁴⁰

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32 ~~Such differences likely reflect the different IgE reactivity profiles due to differences in~~
33 ~~allergen exposure in Africa²⁵.~~

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36 Many studies have revealed an important role of histamine, a key downstream effector
37 molecule in allergic reaction, in the outcome of a malaria parasite infection.^{22-24,41-43,41-44,26-30}

38
39 Moreover, reports indicate that components of the innate immune system, including
40 eosinophils, basophils, and mast cells (MCs), could play important roles in the pathogenesis
41 of malaria.^{41,26} Increased levels of histamine in plasma and tissue, derived from basophils and
42 MCs, notably following stimulation by IgE through the high affinity receptor FcεR1, are
43 associated with the severity of disease in humans infected with *P. falciparum* and in animal
44 malaria models.^{23,42,24,43} Chlorpheniramine, a HR1agonist reversed resistance to chloroquine
45 and amodiaquine both *in vivo* and *in vitro*.^{42,27} Moreover, astemizole, another HR1 agonist,
46 was identified as an anti-malarial agent in a clinical drug library screen.^{43,28} Finally, *P.*
47 *falciparum* produces translationally controlled tumor protein, which is a homolog of the
48 mammalian histamine-releasing factor that causes histamine release from human
49 basophils.^{44,29}

Further research

Our results provide the first birth cohort study addressing the link between malaria and allergic diseases. They contribute to a growing body of evidence that the pathologies are related. ISAAC has revealed a steady but significant increase in prevalence rates of asthma and allergic diseases in Africa. Whilst the majority of studies have focused on large cities, there is increasing urbanization throughout Africa, as well as improved access to primary health care in many areas. A key concern for ISAAC is the extent to which such societal evolution will result in an increase in allergic diseases. Increased urbanization in sub-Saharan Africa is changing the epidemiology of malaria and although resulting in a decrease in risk, will result in more severe clinical malaria in older individuals.^{45,46,30-31} Moreover, a large consumption of anti-malarial drugs in the urban areas provides substantial drug pressure fostering, the selection of drug-resistant parasites. Despite the encouraging recent decrease in malaria incidence rates, even in rural areas, an additional significant concern is the extent to which such an increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines^{47,22}, administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials^{48,23} and alleviate the non-infectious consequences of atopy. Clinical intervention studies should be envisaged.

What is already known on this topic

There are several reports of the beneficial effects of anti-histamines for malaria chemoprophylaxis^{22-24,11-13,47,32} as well as our previous work^{8,18} showing that chromosomal regions associated with malaria are also linked to allergy and atopy.¹⁵⁵⁻¹⁷ There are two epidemiological studies showing opposite effects of malaria on atopy.^{209,36,22}

What this study adds

Using a longitudinal malaria study birth cohort, we identified an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred only after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

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9 We are grateful to the villagers of Dielmo for their participation and sustained collaboration
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31 publication.
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36 the study. MH, HB, BG, SB, FDS, and AT were involved in acquisition of the data. CL, AF,
37 OMP, AS and RP contributed to the analysis and interpretation of the data. MH, CL, HB, BG,
38 SB, FDS, AF, AT, LB, OMP, SM, AS and RP critically reviewed the report and approved its final
39 version for submission. All authors had full access to all of the data in the study and can take
40 responsibility for the integrity of the data and the accuracy of the data analysis. MH and RP
41 are guarantors.
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47 Competing interests: All authors have completed the ICMJE uniform disclosure form at
48 www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and
49 declare: no financial relationships with any organisations that might have an interest in the
50 submitted work in the previous three years; no other relationships or activities that could
51 appear to have influenced the submitted work.
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Ethical approval: The allergy study was approved by the Senegalese National Ethics committee (2009/N°46). Renewed approval of the longitudinal malaria study was obtained from the same committee (2006/N°969).

Data sharing: The allergy database will be made available on-line. The longitudinal malaria data set will be made available following discussion with the coordinators of the three Institutes that govern the dataset through contact with the corresponding author.

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Table 1 Classification of Asthma, Allergic rhinoconjunctivitis, Atopic dermatitis and overall Atopic status according to ISAAC questionnaire in children aged 0-14 from a malaria birth cohort. N is total number of children examined and n-malaria represents those for whom malaria data were recorded. F is the number of females and M the number of males.

	N (F/M)	%	n-malaria (F/M)	Formatted: English (U.K.)
Asthma symptoms				Formatted: English (U.K.)
None	153 (73/80)	87.43	125 (59/66)	Formatted: English (U.K.)
Moderate	4 (1/3)	2.29	4 (1/3)	Formatted: English (U.K.)
Severe	18 (6/12)	10.29	14 (4/10)	Formatted: English (U.K.)
Rhinoconjunctivitis symptoms				
None	146 (64/82)	83.43	120 (52/68)	Formatted: English (U.K.)
Moderate	11 (8/3)	6.29	9 (6/3)	Formatted: English (U.K.)
Severe	18 (6/12)	10.29	14 (6/8)	Formatted: English (U.K.)
Atopic dermatitis symptoms				
None	159 (75/84)	90.86	128 (60/68)	Formatted: English (U.K.)
Moderate	11 (1/10)	6.29	11 (1/10)	Formatted: English (U.K.)
Severe	5 (4/1)	2.86	4 (3/1)	Formatted: English (U.K.)
Atopic tendency				
Unlikely	119 (56/63)	68.00	97 (46/51)	Formatted: English (U.K.)
Probable	16 (8/8)	9.14	14 (6/8)	Formatted: English (U.K.)
Highly probable	40 (16/24)	22.86	32 (12/20)	

Table 2 Impact of allergy status on risk of *P. falciparum* clinical episodes. Shown are the *P* values and adjusted Odds Ratios with 95% confidence intervals calculated from the mixed model analyses. Values for the covariables Age (≥ 3.5 years of age compared to < 3.5 years of age), Trimestrial incidence of *P. falciparum* clinical episodes and HbAS (beta-globin sickle cell trait; AS compared to AA) are those from the Asthma model analysis. For clarity significant co-variables are shown in bold.

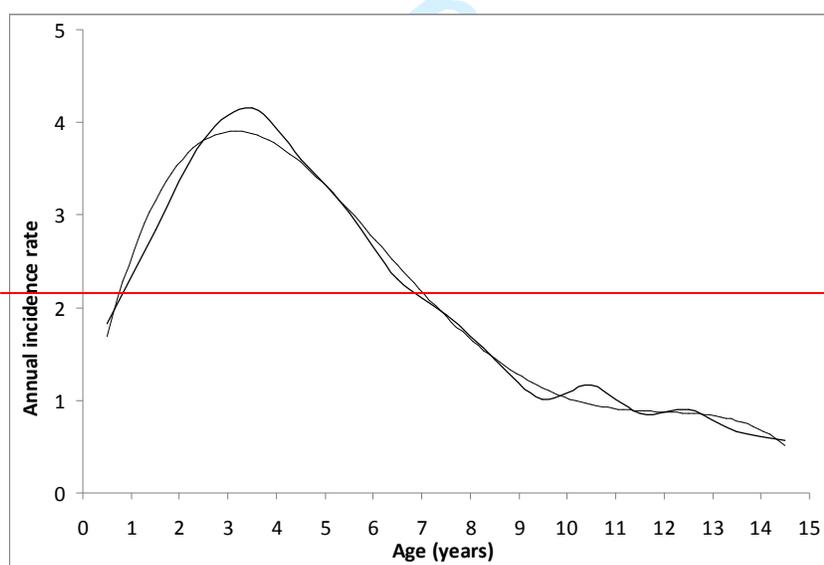
	Age groups < 3.5 years	ORa	95% Confidence Intervals		<i>P</i> value
			Lower	Upper	
Atopy	Both	1.65	1.20	2.26	2.0×10^{-3}
	< 3.5	1.38	0.92	2.08	0.124
	≥ 3.5	2.02	1.39	2.93	2.1×10^{-4}
Asthma	Both	2.12	1.46	3.08	8.0×10^{-5}
	< 3.5	1.50	0.90	2.50	0.122
	≥ 3.5	2.33	1.50	3.61	1.5×10^{-4}
Atopic dermatitis	Both	1.05	0.65	1.70	0.842
	< 3.5	0.84	0.49	1.46	0.539
	≥ 3.5	3.15	1.56	6.33	1.3×10^{-3}
Rhinoconjunctivitis	Both	0.96	0.65	1.41	0.818
	< 3.5	1.05	0.64	1.72	0.853
	≥ 3.5	0.95	0.60	1.52	0.834
Age ≥ 3.5		0.48	0.40	0.57	2.7×10^{-15}
Trimestrial incidence		1.01	1.00	1.01	1.8×10^{-6}
HbAS		0.24	0.12	0.47	3.7×10^{-5}

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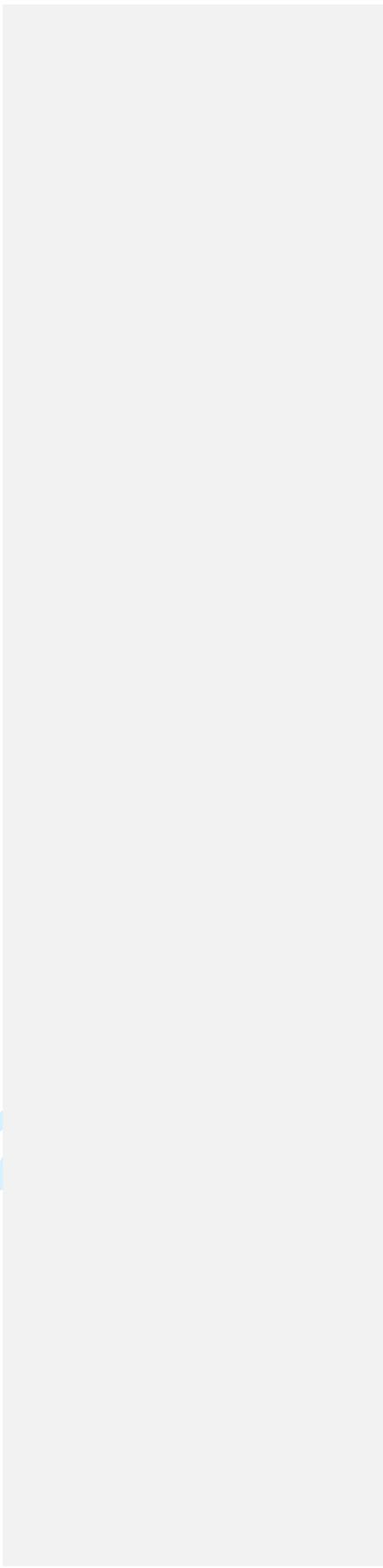
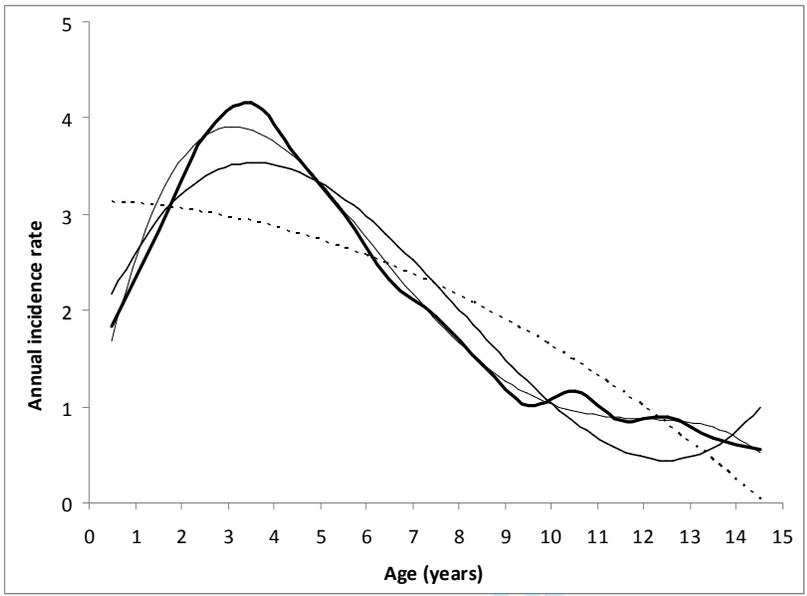
Table 3 Impact of allergy status on the maximum *P. falciparum* parasite density during a clinical malaria episode. Shown are the back-transformed mean parasite densities per microlitre and standard errors (SEM) estimated from the GLMM analyses after taking into account the other co-variables. Significantly different effects are shown in bold for clarity.

Allergic condition	Age groups	Allergic status (No/Yes)	Mean parasite density	SEM	P value
Atopy	Both	N	76.3	13.8	
		Y	131.0	36.4	0.0158
	<3.5	N	114.3	23.7	
		Y	171.1	56.0	0.148
	≥3.5	N	48.4	9.8	
		Y	114.8	37.1	9.5x10⁻⁴
Asthma	Both	N	78.1	14.4	
		Y	148.5	44.3	3.8 x10⁻³
	<3.5	N	114.8	24.3	
		Y	171.9	74.5	0.167
	≥3.5	N	51.3	9.7	
		Y	105.3	41.0	6.2 x10⁻³
Atopic dermatitis	Both	N	82.6	15.0	
		Y	93.9	38.9	0.605
	<3.5	N	122.6	25.5	
		Y	133.9	63.5	0.425
	≥3.5	N	52.3	11.0	
		Y	135.4	70.7	0.014
Rhinconjunctivitis	Both	N	81.5	14.8	
		Y	111.4	39.0	0.570
	<3.5	N	118.8	25.1	
		Y	166.3	69.9	0.537
	≥3.5	N	54.6	11.3	
		Y	80.9	33.7	0.327

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7 **Figure 1** Annual incidence rate of clinical *P. falciparum* episodes per 100 children (~~solid~~-bold line). In order to overcome the fluctuations of the annual incidence rate, we fit second (dotted line), third (dashed line) and fourth (solid line) degree polynomial trend lines to the data (bold line). The corresponding R-squared values are 0.70, 0.91 and 0.99 respectively indicating an accurate fit for third and fourth order polynomials. The inflexion on these two trend lines indicates the onset of acquisition of clinical immunity at approximately 3 to 4 years of age.^a
11 fourth-degree polynomial trendline to the data (dashed line). The corresponding R-squared value is 0.9855 indicating an accurate fit. The inflexion on the trendline indicates the onset of acquisition of clinical immunity at approximately 3-5 years of age.

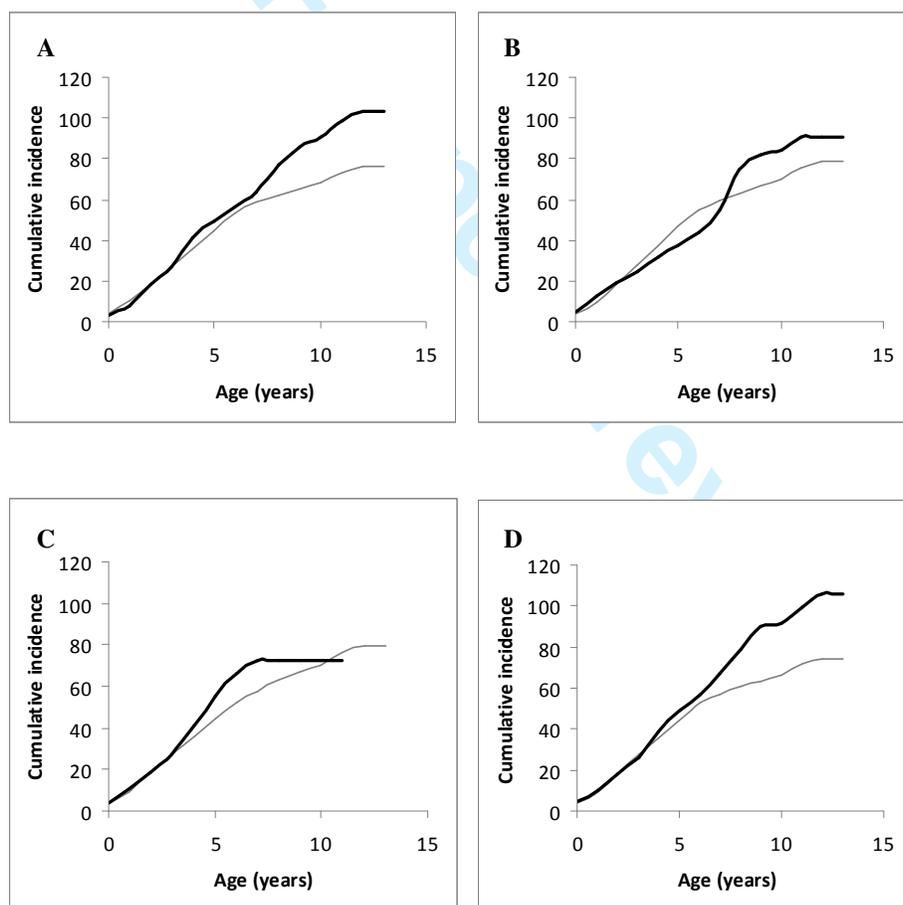


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For peer review only

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7 **Figure 2** Mean cumulative number of *P. falciparum* clinical episodes with age for the (A)
8 Asthma, (B) Rhinoconjunctivitis and (C) Atopic dermatitis classes and overall Atopy class
9 (D) (bold lines) compared to individuals without symptoms of each respective allergy type
10 (~~fine-thin~~ lines). In all cases moderate and severe classes are combined and compared to
11 individuals without allergy symptoms. Note there are no children older than 11 years of age
12 with Atopic dermatitis.
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14



 INSTITUT PASTEUR	Unit of Functional Genetics of Infectious Disease	Coding :	
	ENREGISTREMENT	03/MM/YYYY	Version : 1
ALLERGY MODIFIED ISAAC QUESTIONNAIRE			

Place of study Technician X Technician X Technician X ☎ +	Research Institute responsible Name of Institute Principle investigator Project manager ☎ +
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IDENTIFICATION	Validation zone
Date of questionnaire : <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> dd/mm/yyyy	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> DTEQUE
Name of investigator :	
Name of study supervisor :	
Child :	
First and last name of child :	
Identification code of child :	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> IDENF
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Sex :	<input type="text"/> SEXE
Village/town :	<input type="text"/> VILLAGE
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Identification code of father :	<input type="text"/> <input type="text"/> IDFA
Identification code of mother :	<input type="text"/> <input type="text"/> IDMO
Weight : <input type="text"/> <input type="text"/> <input type="text"/> (kg)	<input type="text"/> <input type="text"/> <input type="text"/> (Kg) WEIGHT
Height : <input type="text"/> <input type="text"/> <input type="text"/> (cm)	<input type="text"/> <input type="text"/> <input type="text"/> (cm) HEIGHT
Mid Upper Arm Circumference: <input type="text"/> <input type="text"/> (cm)	<input type="text"/> (cm) MUAC
Person questioned :	
Last name of person questioned : NAMEPQ
First name of person questioned : LASTNAMEPQ
Relationship to child : Mother <input type="checkbox"/> ₁ Father <input type="checkbox"/> ₂ Brother/Sister <input type="checkbox"/> ₃ Grand-parents <input type="checkbox"/> ₄ Other <input type="checkbox"/> ₅	<input type="text"/> RELCHILD
If other, define : OTHERREL
FACTORS PREDISPOSING ATOPY	
First days of life : Consultation of health records of child and maternity records of mother	
1. How much did your child weigh at birth ?	
<input type="checkbox"/>	
<input type="checkbox"/>	
<input type="checkbox"/>	<input type="text"/> BIRTHWEIGH
2. Until what age did your child breastfeed (exclusively or mixed) ? Corresponds to the age of weaning of child	
<input type="checkbox"/>	
<input type="checkbox"/>	<input type="text"/> AGEWEAN

3. Until what age did your child breastfeed **exclusively** without ever taking other aliments (fruits, vegetables, rice, meat, fish, etc.) or liquids (powdered milk, cow or goats milk, fruit juice, water, etc.) ?
 < 6 months ₁ 6 – 12 mths ₂ 12 – 24 mths ₃ NSP ₉

AGEBREAST

Illness and vaccination : Consultation of health records of child

1. Has your child enfant had the following illnesses?

- Malaria : ₀ No ₁ Yes ₉ NSP
 Tuberculosis treated : ₀ No ₁ Yes ₉ NSP
 Helminths (oxyures, ascaris, taenia, etc.) : ₀ No ₁ Yes ₉ NSP
 Amoeba : ₀ No ₁ Yes ₉ NSP
 Measles : ₀ No ₁ Yes ₉ NSP

MALAR
 TUBTRT
 HEMINTH
 AMOEBA
 MEASLES

2. Against what illnesses is your child vaccinated?

- Yellow fever : ₀ No ₁ Yes ₉ NSP
 Hepatitis B : ₀ No ₁ Yes ₉ NSP
 Measles : ₀ No ₁ Yes ₉ NSP
 Mumps : ₀ No ₁ Yes ₉ NSP
 Rubella : ₀ No ₁ Yes ₉ NSP
 Tuberculosis/BCG : ₀ No ₁ Yes ₉ NSP
 Diphtheria/Tetanus/Pertussis/Poliomyelitis : ₀ No ₁ Yes ₉ NSP
 Typhoid : ₀ No ₁ Yes ₉ NSP
 Meningitis : ₀ No ₁ Yes ₉ NSP
 Haemophilus influenzae type B (HiB) : ₀ No ₁ Yes ₉ NSP

VACFJ
 VACHEPB
 VACMEASLE
 VACMUMPS
 VACRUBEL
 VACTUB
 VACDTCP
 VACTY
 VACMENIN
 VACHIB

Habitation :

1. Which of these animals / insects can be found in the **rooms** where your child lives (today and/or during his first year of life) ?

- Dogs in rooms today : ₀ No ₁ Yes ₉ NSP
 Dogs in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP
 Cats in rooms today : ₀ No ₁ Yes ₉ NSP
 Cats in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP
 Sheep in rooms today : ₀ No ₁ Yes ₉ NSP
 Sheep in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP
 Goats in rooms today : ₀ No ₁ Yes ₉ NSP
 Goats in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP
 Chicken, ducks in rooms today : ₀ No ₁ Yes ₉ NSP
 Chicken, ducks in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP
 Rodents (rats, mice, etc.) in rooms today : ₀ No ₁ Yes ₉ NSP
 Rodents (rats, mice, etc.) in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP
 Cockroaches in rooms today : ₀ No ₁ Yes ₉ NSP
 Cockroaches in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP
 Other in rooms today : ₀ No ₁ Yes ₉ NSP
 Other in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

DOGTODAY
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 CATTODAY
 CAT01YR
 SHEEPTODAY
 SHEEP01YR
 GOATODAY
 GOA01YR
 CHICTODAY
 CHIC01YR
 RODTODAY
 ROD01YR
 COCTODAY
 COC01YR
 OTHTODAY
 OTH01YR

If Others, define :

..... NAMEOTH

2. Which of these animals could be in **contact** with your child **at least once per week**

1	(today and/or during his first year of life) ?		
2	Contact with Dogs today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CDOGTODAY
3	Contact with Dogs 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CDOG01YR
4	Contact with Cats today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CCATODAY
5	Contact with Cats 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CCAT01YR
6	Contact with Sheep today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CSHEEPTODAY
7	Contact with Sheep 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CSHEEP01YR
8	Contact with Goats today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CGOATODAY
9	Contact with Goats 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CGOA01YR
10	Contact with Chicken, Ducks today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CCHICTODAY
11	Contact with Chicken, Ducks 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CCHIC01YR
12	Contact with donkeys, horses today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CHORSTODAY
13	Contact with donkeys, horses 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CHORS01YR
14	Contact with Cows, zébus today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CCOWTODAY
15	Contact with Cows, zébus 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CCOW01YR
16	Contact with Rodents (rats, mice, etc.) today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CRODTODAY
17	Contact with Rodents (rats, mice, etc.) 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> CROD01YR
18	Contact with Other today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> COTHODAY
19	Contact with Other 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> COTH01YR
20	If Others, define :NAMEOTHC
21	3. Which of these aliments are usually stocked in the rooms where your child lives ?		
22	Millet kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> MIL
23	Sorghum kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> SORG
24	Maize kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> MAIZ
25	Rice kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> RICE
26	Wheat kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> WHEA
27	Biscuits, pasta kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> BISCUI
28	Manioc (root, flour) kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> MANIOC
29	Cashew nut, ground nut kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> NUTP
30	Curdled milk kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> MILKCURD
31	Dried leaves (mint, quinquiliba, baobab, etc.) :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> LEAF
32	Other aliments kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> OTHALIM
33	If Others, define :NAMEOTHAL
34	What is the type of roofing of the rooms where your child lives (today and during the first year of life) ?		
35	Corrugated metal roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> RMETTODAY
36	Corrugated metal roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> RMET01YR
37	Thatched roof today:	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> RTHATDAY
38	Thatched roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> RTHAT01YR
39	Wooden roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> RWOOTODAY
40	Wooden roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> RWOO01YR
41	Cement roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> RCEMTODAY
42	Cement roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> RCEM01YR
43	Plaster roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> RPLATODAY
44	Plaster roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> RPLA01YR
45	Other type of roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/> ROTHODAY

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Other type of roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ROTH01YR
If other, define :			NAMEOTHR
4. Which of these objects are in the room where your child sleeps (today and during the first year of life) ?			
Mattress in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MATRTODAY
Mattress in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MATR01YR
Bednet in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	BEDNTODAY
Bednet in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	BEDN01YR
Wardrobe in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WARDTODAY
Wardrobe in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WARD01YR
Chest, trunk in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHESTODAY
Chest, trunk in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHES01YR
Table in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	TABPTODAY
Table in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	TABP01YR
Chair in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHPTODAY
Chair in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHA01YR
Carpet, rug in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CARPTODAY
Carpet, rug in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CARP01YR
Matting in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MATPTODAY
Matting in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MATP01YR
Curtains in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CURTTODAY
Curtains in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CURT01YR
Malagasy fire in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FIRTTODAY
Malagasy fire in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FIR01YR
Other objects in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHOBTTODAY
Other objects in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHOB01YR
If other, define :			NAMEOTHOB
5. On what type of bedding does your child sleep (today and during the first year of life) ?			
Foam mattress today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FMATRTODAY
Foam mattress 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FMATR01YR
Plant fibre mattress (straw, etc.) today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLFMATRTODAY
Plant fibre mattress (straw, etc.) 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLFMATR01YR
Wool mattress today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WOMATRTODAY
Wool mattress 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WOMATR01YR
Feather mattress today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FEATHMTODAY
Feather mattress 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FEATHM01YR
Plastic matting today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLMATTTODAY
Plastic matting 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLMAT01YR
Plant fibre matting (straw, etc.) today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLFMATTTODAY
Plant fibre matting (straw, etc.) 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLFMAT01YR
Other type of bedding today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHBEDTTODAY
Other type of bedding 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHBED01YR
If other, define :			NOMAUTLI
6. Does your child sleep on a pillow ?	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PILLOW
If No, go to question 8			

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If Yes , what type of pillow is it ?			
Foam :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PILLF
Synthetic fibres:	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PILLSYN
Plant fibres (straw, etc.) :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PILLPLF
Feather :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PILLFEATH
Other type of pillow :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHPILL
	If other, define :.....		NAMEOTHPILL
7. Do people smoke in the room where your child lives ?			
Today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	SMOKTODAY
From 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	SMOK01YR
During the pregnancy of the mother :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	SMOKPREG
8. What type of heating and lighting are used in the rooms where your child lives ?			
Heating and lighting by charcoal :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHELCHAR
Heating and lighting by wood :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHELWOO
Lighting by candle :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	LCAND
Lighting by petrol lamp :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	LLAMP
Lighting by flash light :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	LTORCH
Lighting by solar :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	LSOLAR
Other types of heating and lighting:	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHHEL
	If other, define :.....		NAMEOTHHEL
9. Which of the following products are used or stocked in the rooms where you child lives ?			
Insecticide (type Yotox, spirales, etc.) :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	INSECTIC
Deodorants (aerosols) :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	DEODORA
Incense :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	INCENSE
Detergents (type Cotel, etc.) :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	DETERGEN
Petrol, diesel :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PETROL
Other types of products :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHPROD
	If other, define :.....		NAMEOTHPR
<u>Diet :</u>			
1. Has your child had diarrhoea without fever or abdominal pains (colic)			
	following introduction of non-maternal milk in his diet (cow or goat's milk, milk powder) :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>
	after a few months of consuming non-maternal (cow or goat's milk, milk powder) :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>
			DIARINT
			DIARMONTH
2. Currently, how many times, on average, does your child eat the following aliments ?			
<i>The consumption of certain aliments is seasonal.</i>			
Meat :	<input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSMEAT
Fish :	<input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSFISH
Egg :	<input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSEGG
Milk (liquid, powder, curdled) :	<input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSMILK
Banana :	<input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSBANA
Mango :	<input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSMANG
Melon :	<input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSMELON

1	Orange, lime :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSORAN		
2	Potatoes, sweet potatoes :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSPOT		
3	Vegetables :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSVEG		
4	Millet :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSMIL		
5	Sorghum :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSSORG		
6	Maize :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSMSAIS		
7	Rice :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSRICE		
8	Wheat (bread, pasta) :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSWHEA		
9	Nuts (Cashew, ground nut) :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSNUT		
10	Prawns, dried oysters :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSPRAWN		
11	Flavouring cubes Maggi :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSCUBE		
12	Other :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	OTHALCON		
13		If other, define :					<input type="checkbox"/>	NAMEOTHAL	
14		<u>HISTORICAL SYMPTOMATOLOGY OF ALLERGIC REACTIONS</u>							
15		<u>Asthma :</u>							
16	1.	Has a doctor or nurse already said that your child has asthma ?					<input type="checkbox"/>		
17		<input type="checkbox"/> ₀ No <input type="checkbox"/> ₁ Yes <input type="checkbox"/> ₉ NSP					<input type="checkbox"/>	ASTHMA	
18	2.	Has your child already breathed noisily or had whistling in his chest whilst breathing					<input type="checkbox"/>		
19		<input type="checkbox"/> ₀ No <input type="checkbox"/> ₁ Yes <input type="checkbox"/> ₉ NSP					<input type="checkbox"/>	WHISTLING	
20		If No , go directly to question 6							
21	3.	During his first two years of life, has your child already breathed noisily or had whistling in his chest whilst breathing ?					<input type="checkbox"/>		
22		<input type="checkbox"/> ₀ No <input type="checkbox"/> ₁ Yes <input type="checkbox"/> ₉ NSP					<input type="checkbox"/>	WHISTL2YR	
23		If No , go directly to question 6							
24		If Yes , how many times (before 2 years of age) ?					<input type="checkbox"/>		
25		<input type="checkbox"/> ₁ 1time <input type="checkbox"/> ₂ 2times <input type="checkbox"/> ₃ ≥3times <input type="checkbox"/> ₉ NSP					<input type="checkbox"/>	NBWHIS2YR	
26		Between the last two ramadans , has your child already breathed noisily or had whistling in his chest whilst breathing ?						<input type="checkbox"/>	
27		<input type="checkbox"/> ₀ No <input type="checkbox"/> ₁ Yes <input type="checkbox"/> ₉ NSP					<input type="checkbox"/>	WHISTL2RA	
28		If No , go directly to question 5							
29		If Yes , at which moment of the year ?					<input type="checkbox"/>		
30		Rainy season :					<input type="checkbox"/>	WHISTLRS	
31		<input type="checkbox"/> ₀ No <input type="checkbox"/> ₁ Yes <input type="checkbox"/> ₉ NSP					<input type="checkbox"/>	WHISTLDS	
32		Dry season :					<input type="checkbox"/>	WHISTLHT	
33		<input type="checkbox"/> ₀ No <input type="checkbox"/> ₁ Yes <input type="checkbox"/> ₉ NSP					<input type="checkbox"/>		
34		Harvest time :					<input type="checkbox"/>		
35		<input type="checkbox"/> ₀ No <input type="checkbox"/> ₁ Yes <input type="checkbox"/> ₉ NSP					<input type="checkbox"/>		
36		Has the noisy breathing of your child been such that it has prevented him from talking normally?						<input type="checkbox"/>	
37		<input type="checkbox"/> ₀ No <input type="checkbox"/> ₁ Yes <input type="checkbox"/> ₉ NSP					<input type="checkbox"/>	PREVTALK	
38		Has your child already had a rasping cough at night that prevents him from sleeping normally ?						<input type="checkbox"/>	
39		<input type="checkbox"/> ₀ No <input type="checkbox"/> ₁ Yes <input type="checkbox"/> ₉ NSP					<input type="checkbox"/>	TOUSECHE	
40		<u>Rhinitis and allergic conjunctivitis:</u>							
41	1.	Has your child already had problems of a runny nose, or a sensation of a blocked nose, or an itchy nose, or sneezing, or loss of the sense of smell for more than a week ,					<input type="checkbox"/>		

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irrespective of the frequency of these episodes? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHIN1WEEK
2. Has your child already had problems of a runny nose, or a sensation of a blocked nose, or an itchy nose, or sneezing, or loss of the sense of smell more than 5 times in one year , irrespective of the frequency of these episodes? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHIN5FAN
Between the last two ramadans , has your child already had problems of a runny nose, or a sensation of a blocked nose, or an itchy nose, or sneezing, or loss of the sense of smell ? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHIN2RAM
If No , go to question 4		
If Yes , at what moment of the year ?		
Rainy season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHINRS
Dry season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHINDS
Harvest time : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHINHT
3. Has your child already had watery eyes, or itchy eyes, or an allergic limbo-conjunctivitis? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJALER
If No , go directly to question 1 in the section Eczema		
Has your child had, between the last two ramadans , watery eyes, or itchy eyes, or an allergic limbo-conjunctivitis? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJ2RAM
If No , go directly to question 5		
If Yes , at what moment of the year ?		
Rainy season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJRS
Dry season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJDS
Harvest time : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJHT
<u>Eczéma :</u>		
Has your child already had skin problems with dry patches or seeping cracked patches and itching ? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEMA
If No , the questionnaire has finished.		
Between the last two ramadans , has your child had skin problems with dry patches or seeping cracked patches and itching ?? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZE2RAM
If No , go directly to question 3		
If Yes , at what moment of the year ?		
Rainy season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEMARS
Dry season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEMADS
Harvest time : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEMAHT
1. Have these skin problems affected different parts of the body of your child ?		
Scalp : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZESCALP
Face : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEFACE
Around the eyes and ears : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	
Armpits : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEEYEARE

STROBE Statement—checklist of items included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses

Continued on next page

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure <i>Cross-sectional study</i> —Report numbers of outcome events or summary measures
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

Discussion

Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based
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*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.



Asthma and atopic dermatitis are associated with increased risk of clinical Plasmodium falciparum malaria

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Secondary Subject Heading:	Epidemiology, Immunology (including allergy), Infectious diseases, Dermatology
Keywords:	Allergy < THORACIC MEDICINE, Asthma < THORACIC MEDICINE, Epidemiology < INFECTIOUS DISEASES

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Manuscripts

Asthma and atopic dermatitis are associated with increased risk of clinical *Plasmodium falciparum* malaria

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Article summary

Article focus

- Genetic studies suggest a link between susceptibility to allergy and malaria in Africa
- We hypothesize that atopy increases susceptibility to malaria

Key messages

- Results demonstrate an association between asthma, atopic dermatitis and susceptibility to clinical *P. falciparum* episodes.
- Genetic pre-disposition to asthma or atopic dermatitis impairs the acquisition of clinical immunity to malaria.
- Administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Strengths and limitations

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association.

Abstract

Objectives: To assess the impact of atopy and allergy on the risk of clinical malaria.

Design: A clinical and immunological allergy cross-sectional survey in a birth cohort of 175 children from 1 month to 14 years of age followed for up to 15 years in a longitudinal open cohort study of malaria in Senegal. Malaria incidence data were available for 143 of these children (aged 4 months to 14 years of age) for up to 15 years. Mixed model regression analysis was used to determine the impact of allergy status on malaria incidence, adjusting for age, gender, sickle cell trait and force of infection.

Main outcome measures: Asthma, allergic rhinoconjunctivitis and atopic dermatitis status, the number of clinical *Plasmodium falciparum* malaria episodes since birth and associated parasite density.

Results: Twelve percent of the children were classified as asthmatic and ten percent as having atopic dermatitis. These groups had respectively a two-fold (OR 2.12 95% confidence intervals 1.46 to 3.08; $P= 8 \times 10^{-5}$) and three-fold (OR 3.15, 1.56 to 6.33; $P= 1.3 \times 10^{-3}$) increase in the risk of clinical *P. falciparum* malaria once older than the age of peak incidence of clinical malaria (3 to 4 years of age). They also presented with higher *P. falciparum* parasite densities (Asthma: mean 105.3 parasites/ μ L \pm SE 41.0 vs. 51.3 \pm 9.7; $P= 6.2 \times 10^{-3}$; Atopic dermatitis: 135.4 \pm 70.7 vs. 52.3 \pm 11.0; $P=0.014$). There was no effect of allergy on the number of non-malaria clinical presentations. Individuals with allergic rhinoconjunctivitis did not have an increased risk of clinical malaria nor any difference in parasite densities.

Conclusion: These results demonstrate that asthma and atopic dermatitis delay the development of clinical immunity to *P. falciparum*. Despite the encouraging decrease in malaria incidence rates in Africa, a significant concern is the extent to which the increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines, administration to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Introduction

The World Allergy Organization estimates that 40% of the world's population is concerned by allergic diseases.¹ In developing countries where *Plasmodium falciparum* malaria is endemic, prevalence of allergy is significantly lower, but is on the increase.² T helper type 2 (Th2) cells, their related cytokines, IgE, eosinophils and mast cells play a major role in allergic inflammation. Orientation of the immune response towards a Th1 profile is crucial for immunity to intracellular pathogens,³ whereas orientation towards a Th2 profile drives immunity to extracellular pathogens and antigens resulting in class switching giving rise to IgE-producing B cells.⁴ A role of the Th1/Th2 balance in the development of clinical malaria following infection by *P. falciparum* has been suggested by numerous studies.⁵⁻⁷ Whilst it is recognised that acquired anti-parasite immunity is IgG dependent,⁸ parasite-specific IgE also impact upon the clinical outcome of infection. For example, higher IgE but not IgG levels have been observed in patients with cerebral malaria than those with uncomplicated *P. falciparum* infection.⁹ The role of IgE, however, remains unclear.¹⁰

The interplay between infectious agents and allergy is ambiguous. On the one hand, for example, severe respiratory syncytial virus infection in infants increased the risk of allergic rhinoconjunctivitis and allergic asthma.^{11,12} On the other hand, measles,¹³ hepatitis A¹⁴ and tuberculosis¹⁵ seemingly reduce atopy. Although, an atopic condition can increase incidence of disease, such as the case for the skin commensal *Staphylococcus aureus* in patients with atopic dermatitis,¹⁶ an atopic tendency *per se* does not generally lead to increased illness from infectious agents.

Genome wide studies have identified chromosomal regions linked to clinical malaria, all of which overlap with those previously identified to be involved in atopic dermatitis, asthma, atopy and IgE levels,¹⁷⁻¹⁹ suggesting that common mechanisms may be involved in both pathologies.²⁰ Chromosomal region 5q31 that has been repeatedly shown to be associated with control of parasite density and contains a cluster of cytokines, among which IL12B has been previously associated with psoriasis.²¹ The other regions, 13q13-q22, 5p15-p13 and 12q21-q23, contain genes involved in innate immunity, notably the interleukin 7 receptor, and several involved in tumour necrosis factor synthesis [C1q and tumour necrosis factor related protein 3 (C1QTNF3)] and a gene involved in the complement system (C9).²⁰

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3 Several additional lines of evidence support the concept that susceptibility to malaria and
4 atopy may be related to similar immunological defects. In Ethiopia, a history of malaria was
5 associated with atopy.²² A mouse model for human atopic disease was found to be very
6 susceptible to murine malaria and a major locus for atopic disease mapped close to the
7 region controlling parasite density.²³ This region contains several candidate genes that have
8 effects on T-cell function.²³
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14 Moreover, a direct effect of histamine in the malaria pathogenesis has been found using
15 genetic and pharmacological approaches²⁴ and increased levels of histamine are associated
16 with the severity of disease in humans infected with *P. falciparum* and in animal malaria
17 models.^{25,26}
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22 To test the hypothesis that allergy impacts upon clinical *P. falciparum* malaria, we performed
23 a clinical allergy cross-sectional study in the family-based longitudinal cohort from Senegal
24 previously used for the genome linkage study²⁰ and analysed the impact of asthma, atopic
25 dermatitis, allergic rhinoconjunctivitis on the incidence of clinical *P. falciparum* episodes and
26 the maximum parasite density during each episode.
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30 31 32 33 **Methods**

34 35 36 **Population and outcome data**

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38 The malaria research program conducted in Dielmo village in Senegal has been ongoing
39 since 1990 as described elsewhere.²⁷ In brief, between 1990 and 2008, a longitudinal study
40 involving the inhabitants of the village of Dielmo, Senegal, was carried out to identify all
41 episodes of fever. The study design included daily medical surveillance with systematic blood
42 testing of individuals with fever and examination of 200 oil-immersion fields on a thick blood
43 film for malaria parasites (about 0.5 μ L of blood). Each individual was given a unique
44 identification code and details of family ties, occupation, and precise place of residence were
45 recorded on detailed maps of each household with the location of each bedroom. All
46 households were visited daily, absenteeism recorded, and the presence of fever or other
47 symptoms assessed. We systematically recorded body temperature at home three times a
48 week (every second day) in children younger than 5 years, and in older children and adults in
49 cases of suspected fever or fever-related symptoms. In cases of fever or other symptoms,
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3 blood testing was done at the dispensary by finger prick, and we provided detailed medical
4 examination and specific treatment. Parasitologically confirmed clinical malaria episodes
5 were treated according to national guidelines. From 1990 to 2008, four different drug
6 regimens were implemented: Quinine from 1990 to 1994, Chloroquine from 1995 to 2003,
7 Fansidar (sulfadoxine-pyrimethamine) from 2004 to mid-2006 and Artemisinin-based
8 combination therapy (ACT; Amodiaquine- sulfadoxine-pyrimethamine) from mid-2006 to
9 2008.

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11 Parasite positivity was established as follows. Thick blood films were prepared and stained
12 by 3% Giemsa stain. Blood films were examined under an oil immersion objective at x1000
13 magnification by the trained laboratory technicians and 200 thick film fields were examined
14 to count the number of asexual and gametocyte parasite stages. Asexual parasite densities
15 (per μL) were calculated by establishing the ratio of parasites to white blood cells and then
16 multiplying the parasite count by 8,000, the average white blood cell count per μL of blood.

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18 Malaria transmission in Dielmo is intense and perennial. We conducted a cross-sectional
19 survey to estimate the prevalence of symptoms related to allergic diseases among 175
20 children aged from 1 month to 14 years old who were born during the malaria research
21 program.

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23 Both the longitudinal and cross-sectional surveys were approved by the Ministry of Health of
24 Senegal. Informed consent of the volunteers is renewed every year. More specifically for the
25 cross-sectional survey, after informing about the procedures and the purpose of the study,
26 written informed consent was obtained from parents or guardians of children either by
27 signature or by thumbprint on a voluntary consent form written in both French and Wolof,
28 the main local language. Consent was obtained in the presence of the school director, an
29 independent witness.

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31 The family structure (pedigree) was available after a demographic census performed for
32 every volunteer at his adhesion in the project. A verbal interview of mothers or key
33 representatives of the household was used to obtain information on genetic relationships
34 between studied individuals, their children, their parents, and to identify genetic links
35 among the population. The total pedigree comprised 828 individuals, including absent or
36 dead relatives, composed of ten independent families that can be sub-divided into 206
37 nuclear families (father – mother couples with at least one child) with an average of 3.6
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3 children each. Genetically related nuclear families occur because of multiple marriages and
4 marriages among related individuals. Previous typing with microsatellites has enabled the
5 construction of a pedigree based on Identity-by-Descent using MERLIN.^{20,28} The mean
6 coefficient of inbreeding is 0.0008. Newborns since this original genetic analysis were added
7 to the family of the parents in question. The 143 children, with both allergy and malaria
8 data, belonged to 61 nuclear families and comprised 30 singletons, 102 siblings and 11 half-
9 sibs (yielding 55 half-sib pairs). The mean genetic relatedness (by pedigree) of the 143
10 children is 0.0114 (range: 0.0013 to 0.022).

17 *P. falciparum clinical episodes*

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20 *P. falciparum* malaria clinical episode phenotypes analysed were: (i) clinical *P. falciparum*
21 infections treated with anti-malarial therapy and (ii) the highest parasite density during the
22 *P. falciparum* clinical episode. A clinical *P. falciparum* episode was defined as a clinical
23 presentation with fever (axillary temperature $\geq 37.5^{\circ}\text{C}$) and/or other clinical signs suggestive
24 of malaria associated with a thick blood smear positive for *P. falciparum* and that was
25 treated with anti-malarial therapy. Repeated clinical malaria presentations within 15
26 consecutive days were not considered to be independent and were excluded from the
27 analyses, unless there was a negative thick blood smear between two clinical presentations.
28 We also excluded observations in any trimester for which the individual was not present for
29 at least one third of the time.

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31 We calculated the quarterly incidence rate of clinical *P. falciparum* episodes in children
32 below the age of 15 years as the ratio of the total number of clinical *P. falciparum* episodes
33 during the trimester divided by the total number of person-trimesters surveyed. Incidence
34 rate is expressed as cases per 100 person-trimesters (see Supplementary Figure S1). This
35 rate was used in the analysis to approximate the force of infection (exposure level) within
36 the targeted population at the time of a given clinical *P. falciparum* episode.

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38 The total number of clinical presentations per trimester that were not attributable to *P.*
39 *falciparum* was tabulated. Repeated non-malaria presentations within seven consecutive
40 days were not considered to be independent and were excluded.

41 *Allergic diseases and atopic status*

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3 The International Study of Asthma and Allergies in Childhood (ISAAC) diagnostic criteria have
4 been shown to be reproducible, adequate and able to discriminate children with allergic
5 diseases in different areas of the world.² The standardized ISAAC questionnaire originally
6 written in English was translated into French in compliance with ISAAC guidelines²⁹, adapting
7 it to the usual local customs following advice from local clinicians and paediatric
8 allergologists (Acknowledgements and Technical Appendix). The adequacy and reliability of
9 the translated questionnaire had been previously confirmed by a pilot study on 30 randomly
10 selected children in the same community. The questionnaire was completed by specially
11 trained health workers during an oral interview conducted in Wolof with children and their
12 mothers or guardians.
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21 To assess the prevalence of allergic diseases in children, we used the positive and negative
22 predictive values of the ISAAC questionnaire diagnosis criteria developed for subtropical
23 countries.³⁰ Each question was scored according to the medical diagnosis of paediatricians
24 and paediatric allergologists. Positive or negative answers were thus graded on the basis of
25 symptom sensitivity, specificity, frequency, location or early onset. For each allergic disease,
26 three categories of symptom severity, *severe*, *moderate*, and *none*, were defined as follows:
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32 *Asthma – severe* symptoms if the child had “wheezing or whistling in the chest before the
33 age of two years” and “more than three times” or severe enough to “limit his/her speech”;
34 *moderate* symptoms if the child had “wheezing or whistling in the chest before the age of
35 two years” and “in the past 12 months”; and *none* otherwise.
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40 *Allergic rhinoconjunctivitis – severe* symptoms if the child had “sneezing, runny or stuffy nose
41 in the past 12 months” and “more than five times a year”, and “itchy, watery eyes or tropical
42 endemic limboconjunctivitis (TELC) in the past 12 months”; *moderate* symptoms if the child
43 had “sneezing, runny or stuffy nose in the past 12 months”, and “itchy, watery eyes or TELC
44 in the past 12 months”; and *none* otherwise.
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49 *Atopic dermatitis – severe* symptoms if the child had “scaly or exudating, crusted and pruritic
50 patches in the past 12 months” and “affecting any of the following characteristic areas: face,
51 around the ears or eyes, folds of armpits or elbows or groin, behind the knees, under the
52 buttocks”, and “onset of symptoms before the age of two years”; *moderate* symptoms if the
53 child had “scaly or exudating, crusted and pruritic patches in the past 12 months” and
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3 “affecting any of characteristic areas (see above)”, and “onset of symptoms before the age
4 of four years”; and *none* otherwise.

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7 The inter-relationships between variables reflecting the severity of symptoms of the three
8 allergic diseases were used to identify children at high risk of atopy. The *high probability*
9 group was defined by the prevalence of at least one of any *severe* symptoms or two of any
10 *moderate* symptoms. The *probable* group was defined as those with *moderate* symptoms
11 from one of the three allergic diseases and remaining children were classified in the *unlikely*
12 group.

13 14 15 16 17 18 *Helminths*

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20 Helminthic infections are common in this region and are known to modify the clinical course
21 and outcome of both allergic diseases and malaria.^{31,32} We therefore carried out a helminth
22 survey for 91 individuals present during the cross-sectional survey. Diagnosis was performed
23 by stool examination by microscope and by the Kato technique to search for the presence of
24 *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*),
25 whipworm (*Trichuris trichiuria*), *Schistosoma mansoni*, and *Strongyloides stercoralis*.
26 Examination for pinworms (*Enterobius vermicularis*) was performed by the anal scotch-test.
27 An anti-helminthic treatment was proposed for all infested individuals.

28 29 30 31 32 33 34 35 *Immunoglobulin E titres*

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37 Specific IgE titres were measured by ELISA as previously described.³³ A panel of allergens of
38 potential pertinence to the three classes of allergy was used: (i) Salivary gland extracts (SGE)
39 of two mosquito species present in the study cohorts, *Aedes aegypti* and *Anopheles gambiae*
40 *sensu stricto*, and (ii) *P. falciparum* parasite extract were prepared as previously described³¹;
41 (iii) House dust mite spp. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*;
42 (iv) a mix of pollen allergens from five ubiquitous gramineae spp. [Cock’s-foot (*Dactylis*
43 *glomerata*), Timothy grass (*Phleum pratense*), Sweet Vernal grass (*Anthoxanthum*
44 *odoratum*), Perennial ryegrass (*Lolium perenne*), Kentucky Bluegrass (*Poa pratensis*)] (all
45 from Stallergenes, France).

46 47 48 49 50 51 52 53 **Statistical analysis**

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55 Statistical analyses were performed using R version 2.12.0 (The R Foundation for Statistical
56 Computing, Vienna, Austria). To address the effect of allergic status on the risk of clinical *P.*
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3 *falciparum* episodes, we performed Generalized Linear Mixed Models (GLMM) extended to
4 pedigree data using the *pedigreemm* package for R to account for the non-independence of
5 individuals because of family relationships, shared house and for repeated measures from
6 the same individual (Technical Appendix). Correlated individual effects due to familial
7 relationships were taken into account by using the pedigree-based genetic relatedness
8 matrix that contains the genetic covariance among all pairs of individuals in the study cohort
9 and is calculated using the pedigree information.³⁴ Shared house and repeated measures
10 from the same individual were modelled as random effects. All random effects were
11 assumed to be normally distributed, and conditional on these random effects, the
12 dependent variable had: (i) a Binomial distribution when the studied phenotype was the
13 occurrence of a clinical *P. falciparum* episode treated with anti-malarial therapy during a
14 trimester, (ii) a Gaussian distribution when the studied phenotype was the logarithm of the
15 maximum parasite density during a given clinical *P. falciparum* episode, and (iii) a Poisson
16 distribution when the studied phenotype was the number of non-malaria episodes per
17 trimester. The effects of allergy disease classes on these dependent variables were modelled
18 as fixed effects. Allergy classes were reduced to two levels, *Severe* or *moderate vs. none* for
19 analyses of asthma, atopic dermatitis and allergic rhinoconjunctivitis and *high probability vs.*
20 *probable* and *unlikely* for atopic tendency. Co-variables included sickle cell trait³³, gender,
21 number of days present on site during the trimester, trimestrial incidence of *P. falciparum*
22 and age. Age was initially analysed as a continuous covariate. To assess the age-specific
23 effect of allergy, age was categorised into two levels (<3.5 years of age and ≥3.5 years of
24 age, based on the age of peak clinical incidence) and allergy class was nested within age
25 class. The age threshold was varied from 1.5 years to 5.5 years of age and the data re-
26 analysed to assess at which age there was the strongest effect. The association of allergy
27 classes with IgE levels was analysed by box-cox transforming the data and fitting a GLMM
28 with a normal distribution.

51 Results

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54 Of the 205 eligible children aged under 15 years involved in the family-based longitudinal
55 study, 175 (85.4 %) participated in the cross-sectional survey to assess the prevalence of
56 related symptoms of allergic diseases. All eligible children present at the time of the survey
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3 were included; no explicit refusal to participate was recorded. The study cohort was aged
4 from 1 month to 14 years 11 months. The sex-ratio (male/female) was 0.94.
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7 From 1994 until 2008, 143 of the children participating in the cross-sectional survey were
8 present for at least 31 days in any trimester during the study period generating a total of
9 3,093 person-trimesters of presence (Supplementary Table S1). There were 2,065 treated *P.*
10 *falciparum* clinical episodes (per individual: median 11, range 0-47)(Supplementary Table
11 S2). The age peak of incidence of *P. falciparum* episodes occurred at 3 to 4 years of age
12 (Figure 1). There were 1,868 non-malaria episodes (median 12, range 0-37) (Table S2). These
13 non-malaria clinical presentations were associated with headache (38 %), chills (32 %), cough
14 (13 %), vomiting (11 %) and diarrhoea (6 %).
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21 The prevalence of moderate or severe asthma symptoms was respectively 2.3 % and 10.3 %
22 (Table 1). The prevalence of moderate or severe allergic rhinoconjunctivitis symptoms was
23 respectively 6.3 % and 10.3 %. The prevalence of moderate or severe atopic dermatitis
24 symptoms was respectively 6.3 % and 2.9 %. On the basis of symptom severity, an atopic
25 tendency was estimated to be unlikely for 68.0 %, probable for 9.1 % and highly probable for
26 22.9 % of the 175 children. The frequency of each allergy class in children for whom malaria
27 data were available is shown in Table S1.
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34 The risk of treated clinical *P. falciparum* infections was higher for children with high
35 probability of atopy (OR 1.65, 95% confidence intervals 1.20 to 2.26; $P=0.002$) (Table 2), after
36 adjusting for age, sickle cell trait and the exposure level. Gender was not found to be
37 significant. Analysing the impact of atopy in children younger and older than the peak age of
38 clinical incidence (3 to 4 years old), revealed that atopy increased the risk of *P. falciparum*
39 episodes in children at an age greater than 3.5 years (OR 2.02, 1.39 to 2.93; $P=2 \times 10^{-4}$), but
40 not in children of age prior to the peak clinical incidence (OR 1.38, 0.92 to 2.08; $P=0.124$)
41 (Table 2). This increased risk resulted in an ever increasing cumulative number of *P.*
42 *falciparum* episodes with age beyond that of peak clinical incidence (Figure 2. See
43 supplementary Figure S2 for model predictions for comparison).
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52 Analysis by allergy category revealed that asthma (severe or moderate) increases the risk of
53 *P. falciparum* episodes (OR 2.12, 1.46 to 3.08; $P= 8 \times 10^{-5}$) and this again only in children of
54 age greater than 3.5 years old (OR 2.33, 1.50 to 3.61; $P= 1.5 \times 10^{-4}$). Atopic dermatitis
55 increased the risk of clinical malaria in children older (OR 3.15, 1.56 to 6.33; $P= 1.3 \times 10^{-3}$) but
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3 not younger than 3.5 years of age (Table 2). Allergic rhinoconjunctivitis was not associated
4 with increased risk of clinical malaria at any age (Table 2). The impact of atopy, asthma and
5 atopic dermatitis can be clearly seen in the ever-increasing number of cumulative *P.*
6 *falciparum* episodes beyond the age of the onset of clinical immunity in the population, 3.5
7 years of age (Figure 2). There is no difference in the number of clinical malaria episodes prior
8 to this age in individuals with or without an allergic condition. Analysis using different age
9 thresholds (from 1.5 to 5.5 years of age) revealed similar OR for thresholds of 2.5, 3.5 and
10 4.5 years of age. The maximum OR for increased malaria occurred in children older than 4.5
11 years of age and with atopy or atopic dermatitis, whereas for the asthma group it occurred
12 in children after 3.5 years of age (Supplementary Table S3).

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21 There was no impact of any allergic disease on the number of non-malaria episodes by
22 trimester (Supplementary Table S4).

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25 The impact of atopy, asthma and atopic dermatitis on the maximum *P. falciparum* parasite
26 density during a given clinical malaria episode mirrored that of the risk of *P. falciparum*
27 episodes. Parasite density was significantly higher for children with allergic disease older
28 than 3.5 years of age (Table 3 and supplementary Figure S3 for residuals of the fitted model).
29 As the log-transformed data were left skewed, we additionally analysed using box-cox
30 transformation and probit normalization of the data. The results were qualitatively the same
31 (Supplementary text and Figures S4-S8). Allergic rhinoconjunctivitis had no impact on the
32 parasite density (Table 3). Analysis using different age thresholds yielded similar qualitative
33 conclusions as seen with the number of clinical episodes (Table S3).

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42 Individuals with moderate or severe symptoms of atopic dermatitis had significantly higher
43 specific IgE titres against *Ae. aegypti* (P=0.004) and *An. gambiae* SGE (P<0.001). There were
44 no detectable specific anti-*P. falciparum* IgE. Individuals with moderate or severe symptoms
45 of allergic rhinoconjunctivitis did not have significantly higher IgE titres against the tested
46 gramineae (P=0.28), although titres decreased with age (P=0.035). There was also no effect of
47 asthma on IgE titres against the house dust mite spp. tested (*D. farinae* P=0.60 & *D.*
48 *pteronysinus* P=0.27).

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Only five individuals were infested with helminths (two *Ancylostoma*, one *Strongyloides*, one
Trichuris and one *Enterobius*).

Discussion

Principal findings

Establishing the allergic status of children up to the age of 15 years old followed for malaria since birth, revealed an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

Strengths and weaknesses of the study

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association. In addition, although allergy diagnosis for children under 2 years of age is not considered reliable, there were only 15 individuals under 2 at the time of the allergy study of the 143 for whom malaria and allergy data were available.

Meaning of the study

Under intense malaria transmission, after repeated exposure to the parasite, children develop a clinical immunity³⁵, whereby they tolerate elevated parasite densities without showing clinical symptoms. In this cohort, the population mean onset of clinical immunity occurred at 3 to 4 years of age. Although clinical immunity is accompanied by a reduction in parasite density, effective anti-parasite immunity develops much more slowly³⁶ with individuals achieving a state of premunition, whereby they maintain low-grade parasite densities in an asymptomatic state.³⁷ We show here that children with clinically defined asthma or atopic dermatitis have an increased risk of presenting with *P. falciparum* malaria episodes requiring treatment once passing the age of peak clinical incidence. They also had higher parasite density during clinical episodes, suggesting a reduced ability to control parasite replication. The observed increase in clinical incidence of malaria in patients with asthma or atopic dermatitis is not likely to be the result of increased frailty of such individuals; these individuals did not come more frequently to the clinic with non-malaria

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3 symptoms. Our previous genome linkage study identifying chromosomal regions²⁰
4 associated with malaria that overlap with those previously shown to be linked to
5 asthma/atopy suggests that there may be a shared genetic basis to these pathologies rather
6 than any causative effect of one on the other. This is consistent with the increased
7 susceptibility to malaria of mouse atopic models.²³
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10 11 12 **Comparison with other studies**

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14 A previous study in Ethiopia (East Africa) found that a history of malaria (yes/no) increased
15 risk of atopic dermatitis in 306 cases compared to 426 controls as characterized using the
16 ISAAC questionnaire.²² The only other epidemiological study that has previously examined
17 the link between malaria and atopy³⁸ also interpreted the result from the perspective of the
18 impact of malaria on atopy. They examined the re-infection rate with *P. falciparum* over a 5-
19 year period in 91 children that were subsequently classified as atopic or not using skin prick
20 tests (SPT) with house dust mite antigen. Their conclusion was that, as with measles¹³ and
21 tuberculosis¹⁵, malaria infection reduces atopy. However, the study lacked previous infection
22 data since birth of the participating individuals and focussed on atopy as determined by SPT
23 against a single allergen. The case-control study of atopic dermatitis risk factors cited above
24 found no overall association between allergen skin sensitization and atopic dermatitis. We
25 also found no evidence of increased IgE titres against house dust mites in the asthmatic or
26 atopic dermatitis groups or against grass pollen in individuals with allergic
27 rhinoconjunctivitis. Such differences likely reflect the different IgE reactivity profiles due to
28 differences in allergen exposure in Africa.³⁹ There was no evidence of anti-parasite IgE in this
29 cohort of children. We previously showed that circulating anti-parasite IgE titres were
30 strongly positively correlated with anti-mosquito saliva IgE, but became undetectable
31 following malaria exposure, potentially being bound to effector cells.³³ Only mosquito saliva,
32 a known major local allergen, induced a specific IgE response at significantly higher titres in
33 individuals with atopic dermatitis.
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50 Although the immune effectors of clinical immunity are still poorly defined, there is strong
51 evidence that acquired anti-parasite immunity is IgG-dependent⁸ and cytophilic
52 immunoglobulins (IgG1 & IgG3), which are capable of eliminating the parasites by
53 opsonisation and/or by Antibody Dependent Cellular Immunity play an important role in
54 premunition.³⁷ The higher parasite density during symptomatic episodes observed in the
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3 asthma group suggests impaired development of acquired immunity. Impaired acquisition of
4 immunity to malaria in children with asthma or atopic dermatitis may stem from their
5 imbalanced Th1/Th2 response. Indeed, an atopic state may generate a tendency to develop
6 a Th2 type immune response to *P. falciparum*. Dendritic cells that are oriented to a Th2
7 phenotype are more susceptible to orient the acquired immune response towards a Th2
8 profile.⁴⁰ Orientation of the immune response towards a Th2 profile by asthma or atopic
9 dermatitis would result in a poor Th1 response (and hence development of protective IgG
10 immunoglobulins), considered to be the dominant arm of the immune response enabling
11 resistance to infectious disease in children.⁴¹

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20 Many studies have revealed an important role of histamine, a key downstream effector
21 molecule in allergic reaction, in the outcome of a malaria parasite infection.^{24-26,42-45}
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23 Moreover, reports indicate that components of the innate immune system, including
24 eosinophils, basophils, and mast cells (MCs), could play important roles in the pathogenesis
25 of malaria.⁴² Increased levels of histamine in plasma and tissue, derived from basophils and
26 MCs, notably following stimulation by IgE through the high affinity receptor FcεR1, are
27 associated with the severity of disease in humans infected with *P. falciparum* and in animal
28 malaria models.^{25,26} Chlorpheniramine, a HR1agonist reversed resistance to chloroquine and
29 amodiaquine both *in vivo* and *in vitro*.⁴³ Moreover, astemizole, another HR1 agonist, was
30 identified as an anti-malarial agent in a clinical drug library screen.⁴⁴ Finally, *P. falciparum*
31 produces translationally controlled tumor protein, which is a homolog of the mammalian
32 histamine-releasing factor that causes histamine release from human basophils.⁴⁵

41 Further research

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43 Our results provide the first birth cohort study addressing the link between malaria and
44 allergic diseases. They contribute to a growing body of evidence that the pathologies are
45 related. ISAAC has revealed a steady but significant increase in prevalence rates of asthma
46 and allergic diseases in Africa. Whilst the majority of studies have focused on large cities,
47 there is increasing urbanization throughout Africa, as well as improved access to primary
48 health care in many areas. A key concern for ISAAC is the extent to which such societal
49 evolution will result in an increase in allergic diseases. Increased urbanization in sub-Saharan
50 Africa is changing the epidemiology of malaria and although resulting in a decrease in risk,
51 will result in more severe clinical malaria in older individuals.^{46,47} Moreover, a large
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3 consumption of anti-malarial drugs in the urban areas provides substantial drug pressure
4 fostering, the selection of drug-resistant parasites. Despite the encouraging recent decrease
5 in malaria incidence rates, even in rural areas, an additional significant concern is the extent
6 to which such an increase in allergy will exacerbate the burden of malaria. Given the
7 demonstrated anti-parasitic effect of anti-histamines,⁴⁸ administration of anti-histamines to
8 atopic children will likely reduce the burden of clinical malaria in these children, increase the
9 efficacy of first-line treatment anti-malarials⁴⁹ and alleviate the non-infectious consequences
10 of atopy. Clinical intervention studies should be envisaged.

17 18 **What is already known on this topic**

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20 There are several reports of the beneficial effects of anti-histamines for malaria
21 chemoprophylaxis^{24-26,48} as well as our previous work²⁰ showing that chromosomal regions
22 associated with malaria are also linked to allergy and atopy.¹⁷⁻¹⁹ There are two
23 epidemiological studies showing opposite effects of malaria on atopy.^{22,38}

27 28 **What this study adds**

29
30 Using a longitudinal malaria study birth cohort, we identified an association of asthma and
31 atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the
32 increase in risk of malaria associated with these allergic conditions occurred only after the
33 peak clinical incidence of disease in the population, suggesting that they delay the
34 development of clinical immunity to malaria.

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13
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21
22

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29
30

31
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35
36

37
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Table 1 Classification of Asthma, Allergic rhinoconjunctivitis, Atopic dermatitis and overall Atopic status according to ISAAC questionnaire in children aged 0-14 from a malaria birth cohort. N is total number of children examined and n-malaria represents those for whom malaria data were recorded. F is the number of females and M the number of males.

	N (F/M)	%	n-malaria (F/M)
Asthma symptoms			
None	153 (73/80)	87.43	125 (59/66)
Moderate	4 (1/3)	2.29	4 (1/3)
Severe	18 (6/12)	10.29	14 4/10)
Rhinoconjunctivitis symptoms			
None	146 (64/82)	83.43	120 (52/68)
Moderate	11 (8/3)	6.29	9(6/3)
Severe	18 (6/12)	10.29	14 (6/8)
Atopic dermatitis symptoms			
None	159 (75/84)	90.86	128 (60/68)
Moderate	11 (1/10)	6.29	11 (1/10)
Severe	5 (4/1)	2.86	4 (3/1)
Atopic tendency			
Unlikely	119 (56/63)	68.00	97 (46/51)
Probable	16 (8/8)	9.14	14 (6/8)
Highly probable	40 (16/24)	22.86	32 (12/20)

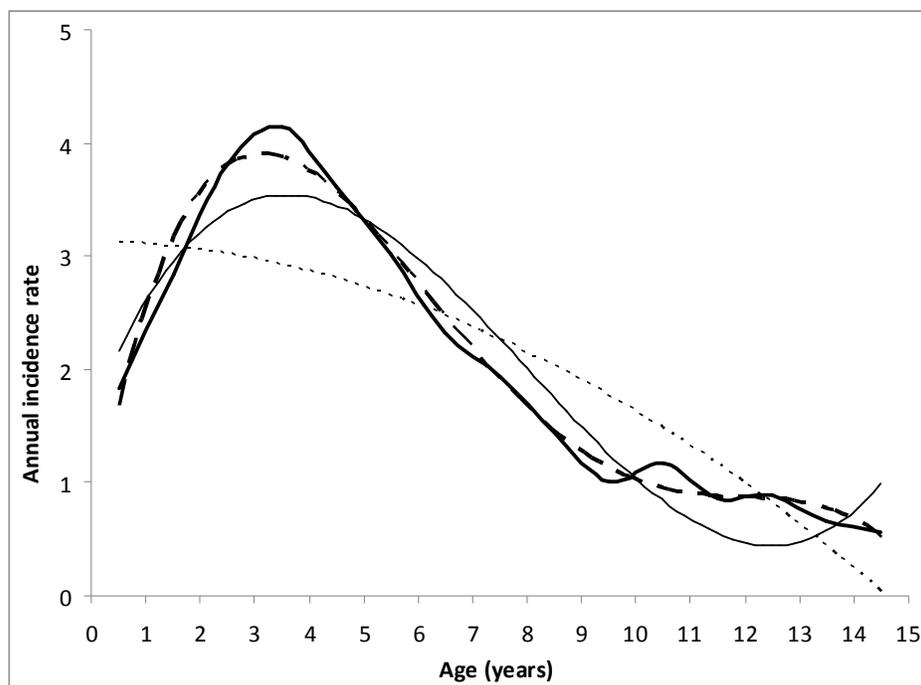
Table 2 Impact of allergy status on risk of *P. falciparum* clinical episodes. Shown are the *P* values and adjusted Odds Ratios with 95% confidence intervals calculated from the mixed model analyses. Values for the covariables Age (≥ 3.5 years of age compared to < 3.5 years of age), Trimestrial incidence of *P. falciparum* clinical episodes and HbAS (beta-globin sickle cell trait; AS compared to AA) are those from the Asthma model analysis. For clarity significant co-variables are shown in bold.

	Age groups < 3.5 years $>$	ORa	95% Confidence Intervals		<i>P</i> value
			Lower	Upper	
Atopy	Both	1.65	1.20	2.26	2.0×10^{-3}
	< 3.5	1.38	0.92	2.08	0.124
	≥ 3.5	2.02	1.39	2.93	2.1×10^{-4}
Asthma	Both	2.12	1.46	3.08	8.0×10^{-5}
	< 3.5	1.50	0.90	2.50	0.122
	≥ 3.5	2.33	1.50	3.61	1.5×10^{-4}
Atopic dermatitis	Both	1.05	0.65	1.70	0.842
	< 3.5	0.84	0.49	1.46	0.539
	≥ 3.5	3.15	1.56	6.33	1.3×10^{-3}
Rhinoconjunctivitis	Both	0.96	0.65	1.41	0.818
	< 3.5	1.05	0.64	1.72	0.853
	≥ 3.5	0.95	0.60	1.52	0.834
Age ≥ 3.5		0.48	0.40	0.57	2.7×10^{-15}
Trimestrial incidence		1.01	1.00	1.01	1.8×10^{-6}
HbAS		0.24	0.12	0.47	3.7×10^{-5}

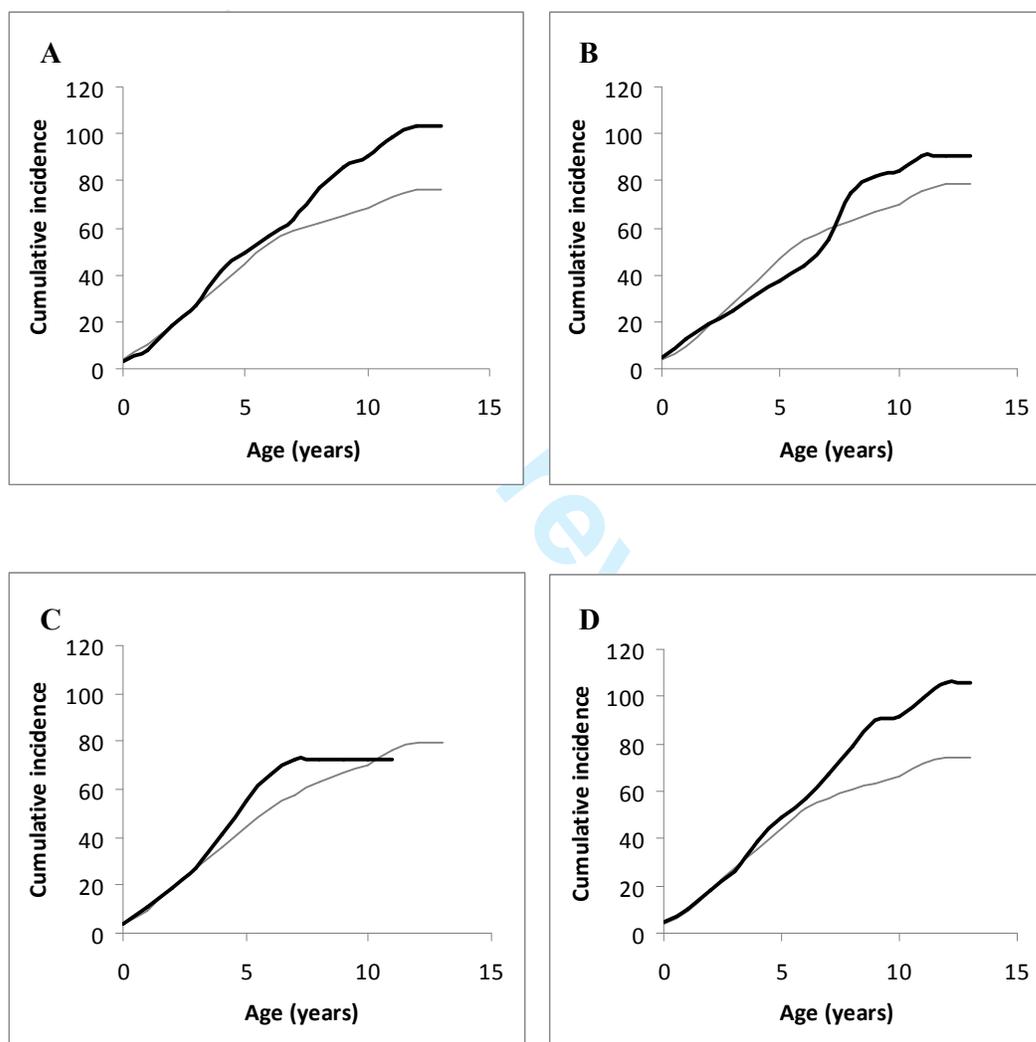
Table 3 Impact of allergy status on the maximum *P. falciparum* parasite density during a clinical malaria episode. Shown are the back-transformed mean parasite densities per microlitre and standard errors (SEM) estimated from the GLMM analyses after taking into account the other co-variables. Significantly different effects are shown in bold for clarity.

Allergic condition	Age groups	Allergic status (No/Yes)	Mean parasite density	SEM	<i>P</i> value	
Atopy	Both	N	76.3	13.8		
		Y	131.0	36.4	0.0158	
	<3.5	N	114.3	23.7		
		Y	171.1	56.0	0.148	
		≥3.5	N	48.4	9.8	
			Y	114.8	37.1	9.5x10⁻⁴
Asthma	Both	N	78.1	14.4		
		Y	148.5	44.3	3.8 x10⁻³	
	<3.5	N	114.8	24.3		
		Y	171.9	74.5	0.167	
		≥3.5	N	51.3	9.7	
			Y	105.3	41.0	6.2 x10⁻³
Atopic dermatitis	Both	N	82.6	15.0		
		Y	93.9	38.9	0.605	
	<3.5	N	122.6	25.5		
		Y	133.9	63.5	0.425	
		≥3.5	N	52.3	11.0	
			Y	135.4	70.7	0.014
Rhinoconjunctivitis	Both	N	81.5	14.8		
		Y	111.4	39.0	0.570	
	<3.5	N	118.8	25.1		
		Y	166.3	69.9	0.537	
		≥3.5	N	54.6	11.3	
			Y	80.9	33.7	0.327

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3 **Figure 1** Annual incidence rate of clinical *P. falciparum* episodes per 100 children (bold
4 line). In order to overcome the fluctuations of the annual incidence rate, we fit second (dotted
5 line), third (dashed line) and fourth (solid line) degree polynomial trend lines to the data (bold
6 line). The corresponding R-squared values are 0.70, 0.91 and 0.99 respectively indicating an
7 accurate fit for third and fourth order polynomials. The inflexion on these two trend lines
8 indicates the onset of acquisition of clinical immunity at approximately 3 to 4 years of age.
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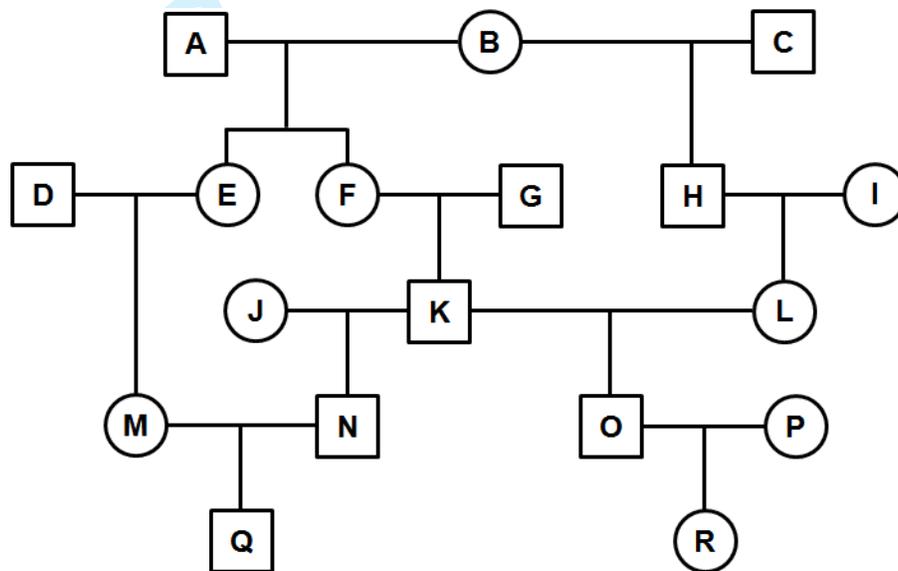
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3 **Figure 2** Mean cumulative number of *P. falciparum* clinical episodes with age for the (A)
4 Asthma, (B) Rhinoconjunctivitis and (C) Atopic dermatitis classes and overall Atopy class
5 (D) (bold lines) compared to individuals without symptoms of each respective allergy type
6 (thin lines). In all cases moderate and severe classes are combined and compared to
7 individuals without allergy symptoms. Note there are no children older than 11 years of age
8 with Atopic dermatitis.
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Pedigree-based genetic relatedness

The Genetic covariance between two individuals can be computed using the pedigree information. For individuals A and B, a given pair in a pedigree, the genetic covariance is computed as $r(A,B) = 2 \times \text{coancestry}(A,B)$ where the *coancestry* between A and B is calculated referring to the method presented by Falconer and Mackay in 1996 (Falconer and Mackay 1996): $\text{coancestry}(A,B) = \sum_p (1/2)^{n(p)} \times (1 + I_{\text{Common Ancestor}})$ where p is the number of paths in the pedigree linking A and B, $n(p)$ the number of individuals (including A and B) for each path p and I_X is the *inbreeding* coefficient of X also equal to the *coancestry* between the two parents of X, I_X is set to 0 if X is a founder.

Illustration: Consider, as an example, the pedigree below containing 18 individuals named {A, B, ..., R} for the calculation of genetic covariance's.



Pedigree structure.

The genetic relatedness between individuals N and O is equal to 0.266. This value is calculated as followed:

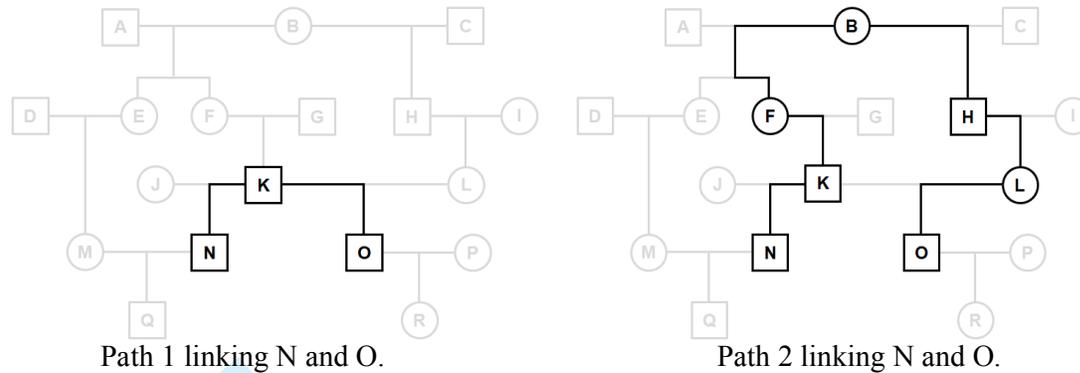
The number of paths linking N and O from the pedigree structure above is $p = 2$.

As illustrated below:

- **Path 1** contains $n(1) = 3$ individuals {N, K, O} with K as the common ancestor. Inbreeding coefficient of K, I_K , is the *coancestry* between the two parents of K (F and G) and is null because F and G are not genetically linked.
- **Path 2** contains $n(2) = 7$ individuals {N, K, F, B, H, L, O} with B as the common ancestor. Inbreeding coefficient of B, I_B , is null because B is a founder.

Therefore, genetic relatedness between individuals N and O is:

$$\begin{aligned}
 &= 2 \times (0.5^{n(1)} \times (1 + I_K) + 0.5^{n(2)} \times (1 + I_B)) \\
 &= 2 \times (0.5^3 \times (1 + 0) + 0.5^7 \times (1 + 0)) = 0.266
 \end{aligned}$$



Defining an equivalent model design where individual effects are independent using the genetic relatedness matrix:

Let us rename $Y^* = l(\mu)$. Y^* can be considered as a linearization of the phenotype through the link function l . The expected mean of Y^* and the variance of Y^* are:

- (i) $E(Y^*) = E(X\beta + Z\gamma + \varepsilon)$
 $= E(X\beta) + E(Z\gamma) + E(\varepsilon) = X \times E(\beta) + Z \times E(\gamma) + E(\varepsilon)$
 $= X\beta$ (asymptotically).
- (ii) $\text{Var}(Y^*) = \text{Var}(X\beta + Z\gamma + \varepsilon)$
 $= \text{Var}(Z\gamma + \varepsilon)$ (as $X\beta$ is the fixed part, thus has variance equal to 0)
 $= \text{Var}(Z\gamma) + \text{Var}(\varepsilon)$ (as γ and ε are independent)
 $= Z \times \text{Var}(\gamma) \times Z^T + \text{Var}(\varepsilon)$ (Z^T is the transpose of Z)
 $= Z(A\sigma_g^2)Z^T + I\sigma_r^2$
 $= ZAZ^T\sigma_g^2 + I\sigma_r^2$

If individuals were independent, i.e. $A = I_N$, variance of Y^* could be expressed as $ZZ^T\sigma_g^2 + I\sigma_r^2$. However, using linear algebra theory by the method “Cholesky decomposition of a matrix”, we can show that there is an equivalent expression of the variance of Y^* corresponding to the modeling of data from independent individuals, having γ^* as an equivalent vector of random effects and Z^* an equivalent design matrix relating γ^* to Y^* so that:

$\text{Var}(Y^*) = Z^*(I\sigma_g^2)Z^{*T} + I\sigma_r^2$. $I\sigma_g^2$ is then the covariance matrix of the equivalent independent random individual effects γ^* .

Theorem: Cholesky decomposition of a matrix

If A is a symmetric positive-definite matrix, there is a triangular matrix L so that A can be written as $A = LL^T$. L can be seen as the “square root” of the matrix A .

Note that the genetic relatedness matrix A computed using the pedigree information (Falconer and Mackay 1996) is a positive-definite matrix, unless identical twins are in the pedigree in which case it would be positive semi-definite.

Equivalent model with independent random effects: We set $A = LL^T$ then:

$$\begin{aligned} \text{Var}(Y^*) &= Z(A\sigma_g^2)Z^T + I\sigma_r^2 \\ &= Z(LL^T\sigma_g^2)Z^T + I\sigma_r^2 \end{aligned}$$

$$\begin{aligned}
 &= ZLL^T Z^T \sigma_g^2 + I\sigma_r^2 \\
 &= (ZL)(ZL)^T \sigma_g^2 + I\sigma_r^2 \\
 &= (Z^*)(Z^*)^T \sigma_g^2 + I\sigma_r^2 \quad (\text{where we set } Z^* = ZL)
 \end{aligned}$$

Then, if we define $\gamma^* = L^{-1}\gamma$, we can rewrite the model as:

$$Y^* = X\beta + Z^*\gamma^* + \varepsilon \quad (\text{because } Z\gamma = Z(LL^{-1})\gamma = (ZL)(L^{-1}\gamma) = Z^*\gamma^*),$$

and the γ_i^* are independent, in other terms $\text{Var}(\gamma^*) = I\sigma_g^2$, as demonstrated below:

We assumed that $\gamma \sim N(0, A\sigma_g^2)$. Then $\gamma^* = L^{-1}\gamma$ is also distributed as a multivariate Normal with mean $E(\gamma^*) = L^{-1}E(\gamma) = L^{-1} \times 0 = 0$ and variance:

$$\begin{aligned}
 \text{Var}(\gamma^*) &= (L^{-1}) \times \text{Var}(\gamma) \times (L^{-1})^T \\
 &= (L^{-1}) \times A\sigma_g^2 \times (L^{-1})^T = (L^{-1})LL^T(L^{-1})^T \sigma_g^2 \\
 &= (L^{-1}L)(L^{-1}L)^T \sigma_g^2 \\
 &= I\sigma_g^2
 \end{aligned}$$

The random effects are now independent and then the classical mixed model assuming independence between levels (here individuals) is applied, and the estimate of fixed effects obtained are fine, i.e. corrected for genetic relationships.

References

Falconer DS, Mackay TFC (1996) Introduction to Quantitative Genetics. 4th Edn. London: Longman.

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Supplementary Tables

Table S1 Number of person-trimesters contributed by number of children by age class and the number who had severe/moderate allergy symptoms, for whom malaria data were also available. AS – Asthma, AD – Atopic dermatitis, RC – Rhinoconjunctivitis. Shown also are the numbers of these individuals suffering from two or all three allergy conditions.

Age group	N° person-trimesters	N° people	AS	AD	RC	AS+AD	AS+RC	AD+RC	AS+AD+RC
]1	7	6	1	2	2	0	1	0	0
]2	21	9	0	1	3	0	0	0	0
]3	48	11	1	1	2	0	0	1	0
]4	119	12	1	2	3	0	0	1	0
]5	102	11	3	4	3	2	1	2	1
]6	125	11	1	1	0	0	0	0	0
]7	303	11	1	2	1	1	0	0	0
]8	340	12	1	1	1	1	0	0	0
]9	362	10	2	0	1	0	1	0	0
]10	610	17	1	0	3	0	0	0	0
]11	77	4	2	1	0	0	0	0	0
]12	484	16	3	0	3	0	1	0	0
]13	390	10	1	0	0	0	0	0	0
]14	105	3	0	0	1	0	0	0	0
Total	3093	143	18	15	23	4	4	4	1

Table S2 Summary of total number of person-trimesters with non-malaria and symptomatic *P. falciparum* clinical presentations and total number of non-malaria episodes according to age class. Given are the number of people contributing to each type of presentation.

	Age group (years)	
	<3·5	≥3·5
Total person-trimesters	1283	1810
People	126	113
Total <i>P. falciparum</i> symptomatic trimesters	963	1102
People	114	108
Total non-malaria episodes	754	1114
People	123	109

Table S3 Effect of changing age threshold on impact of allergy on the risk of clinical malaria and concomitant parasite density. Given are Odds Ratio with 95% confidence intervals, for clinical malaria episodes and the beta coefficient and standard error for parasite density. Corresponding P values are also given. Values are from the nested GLMM analyses.

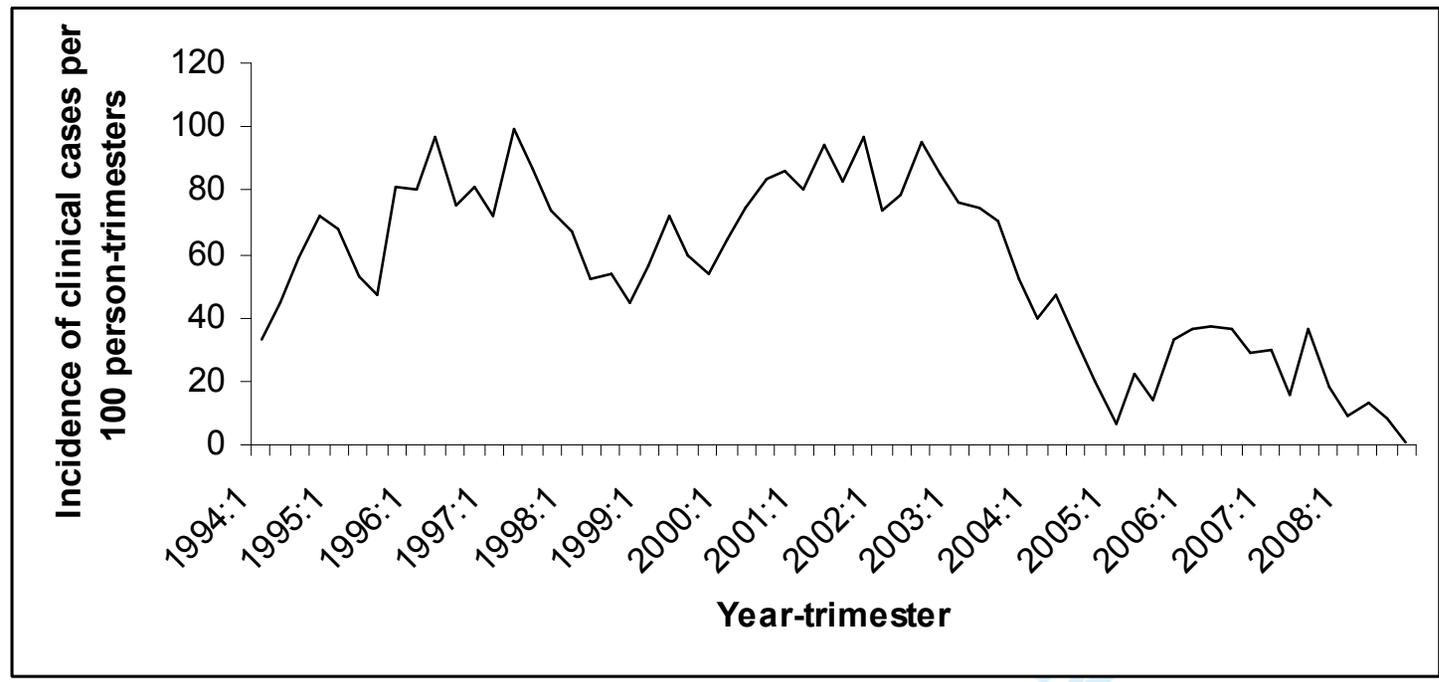
A. Malaria episodes							B. Parasite density				
Age cut-off (years)	OR	95% CI	P value	OR	95% CI	P value	Age cut-off	beta coeff (se)	P value	beta coeff (se)	P value
		above threshold			below threshold		Atopy	above threshold		below threshold	
1.5	1.80	1.25-2.59	1.7x10 ⁻³	1.57	0.85-2.89	0.15	1.5	0.70 (0.27)	9.2x10 ⁻³	0.54 (0.35)	0.12
2.5	2.00	1.39-2.88	2.0x10 ⁻⁴	1.23	0.76-1.99	0.40	2.5	0.79 (0.26)	2.6x10 ⁻³	0.35 (0.29)	0.23
3.5	2.02	1.39-2.93	2.1x10 ⁻⁴	1.38	0.92-2.08	0.12	3.5	0.85 (0.26)	9.5x10 ⁻⁴	0.37 (0.26)	0.15
4.5	2.10	1.42-3.10	1.6x10 ⁻⁴	1.41	0.98-2.04	0.063	4.5	0.87 (0.25)	6.9x10 ⁻⁴	0.40 (0.23)	0.09
5.5	1.64	1.07-2.52	0.02	1.67	1.17-2.37	0.004	5.5	0.73 (0.27)	7.4x10 ⁻³	0.48 (0.22)	3.4x10 ⁻³
Asthma							Asthma				
1.5	1.98	1.29-3.03	1.8x10 ⁻³	1.46	0.69-3.19	0.34	1.5	0.66 (0.31)	0.03	0.30 (0.44)	0.48
2.5	2.30	1.49-3.55	1.6x10 ⁻⁴	1.15	0.63-2.09	0.65	2.5	0.78 (0.30)	0.01	0.26 (0.36)	0.48
3.5	2.33	1.50-3.61	1.5x10 ⁻⁴	1.50	0.90-2.50	0.12	3.5	0.82 (0.30)	6.2x10 ⁻³	0.43 (0.31)	0.17
4.5	2.30	1.48-3.59	2.4x10 ⁻⁴	1.76	1.11-2.80	0.017	4.5	0.81 (0.29)	5.8x10 ⁻³	0.56 (0.28)	0.049
5.5	1.98	1.22-3.22	0.006	2.06	1.33-3.18	0.0011	5.5	0.72 (0.31)	0.02	0.62 (0.27)	0.02
Atopic Dermatitis							Atopic Dermatitis				
1.5	2.05	1.18-3.56	0.01	0.91	0.42-1.97	0.80	1.5	0.80 (0.37)	0.03	0.72 (0.46)	0.12
2.5	2.49	1.36-4.57	3.1x10 ⁻³	0.82	0.44-1.53	0.53	2.5	0.77 (0.38)	0.044	0.52 (0.39)	0.19
3.5	3.15	1.56-6.33	1.3x10 ⁻³	0.84	0.49-1.46	0.54	3.5	0.99 (0.40)	0.014	0.28 (0.35)	0.42
4.5	3.79	1.61-8.92	2.3x10 ⁻³	0.94	0.57-1.57	0.82	4.5	0.98 (0.47)	0.036	0.29 (0.32)	0.37
5.5	1.33	0.47-3.77	0.59	1.19	0.73-1.96	0.49	5.5	0.26 (0.61)	0.67	0.38 (0.31)	0.22
Rhinoconjunctivitis							Rhinoconjunctivitis				
1.5	1.04	0.66-1.62	0.88	1.01	0.51-2.01	0.98	1.5	0.36 (0.32)	0.27	0.18 (0.41)	0.66
2.5	1.01	0.64-1.61	0.96	0.96	0.55-1.68	0.89	2.5	0.28 (0.33)	0.40	0.25 (0.35)	0.48
3.5	0.95	0.60-1.52	0.83	1.05	0.64-1.72	0.85	3.5	0.31 (0.32)	0.33	0.19 (0.31)	0.54
4.5	0.87	0.54-1.42	0.59	1.06	0.68-1.66	0.79	4.5	0.20 (0.32)	0.53	0.22 (0.28)	0.44
5.5	0.81	0.48-1.36	0.43	1.07	0.70-1.64	0.74	5.5	0.10 (0.33)	0.75	0.23 (0.27)	0.39

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4 **Table S4 Frequency of non-malaria episodes (number of days of presence divided by number of non-malaria episodes) according to allergic status**
5 **and age group.** The *P* value is that from the GLMM analyses of the effect of allergic status by age group on the number of non-malaria episodes per person-
6 trimester.
7

Allergic condition	Allergic status (No/Yes)	Age group (years)		<i>P</i> value
		<3·5	>3·5	
Atopy	N	78·2	85·9	0·105
	Y	87·2	102·6	
Asthma	N	79·6	87·3	0·319
	Y	82·5	100·2	
Atopic dermatitis	N	80·9	88·2	0·323
	Y	73·4	101·9	
Rhinoconjunctivitis	N	77·9	88·3	0·167
	Y	94·9	91·8	

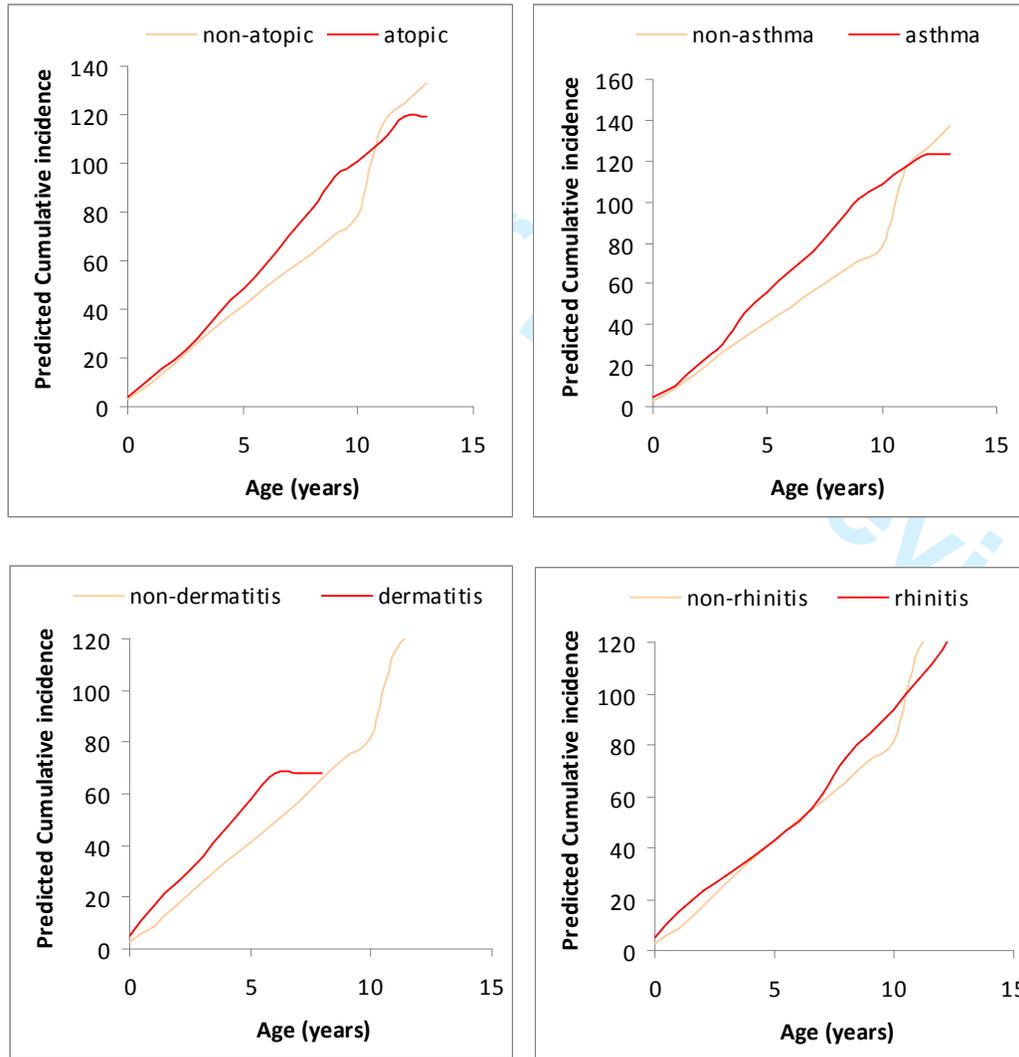
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Figure S1. Incidence of clinical cases per 100 person-trimesters in children under 15 years of age.



Only

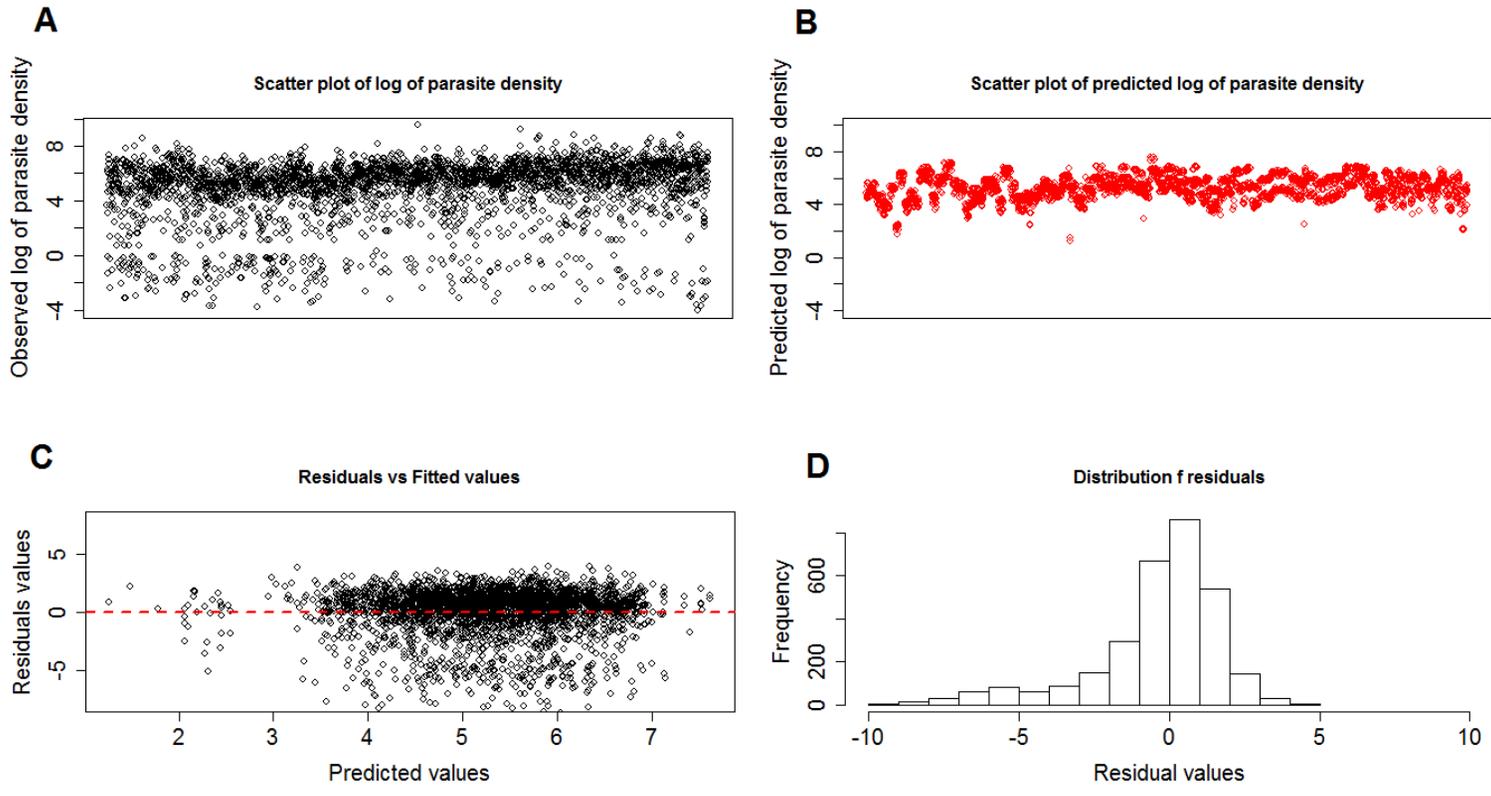
Figure S2. Cumulative incidence of clinical cases according to allergy class predicted by the statistical model.



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Figure S3. Graphical control model for parasite density

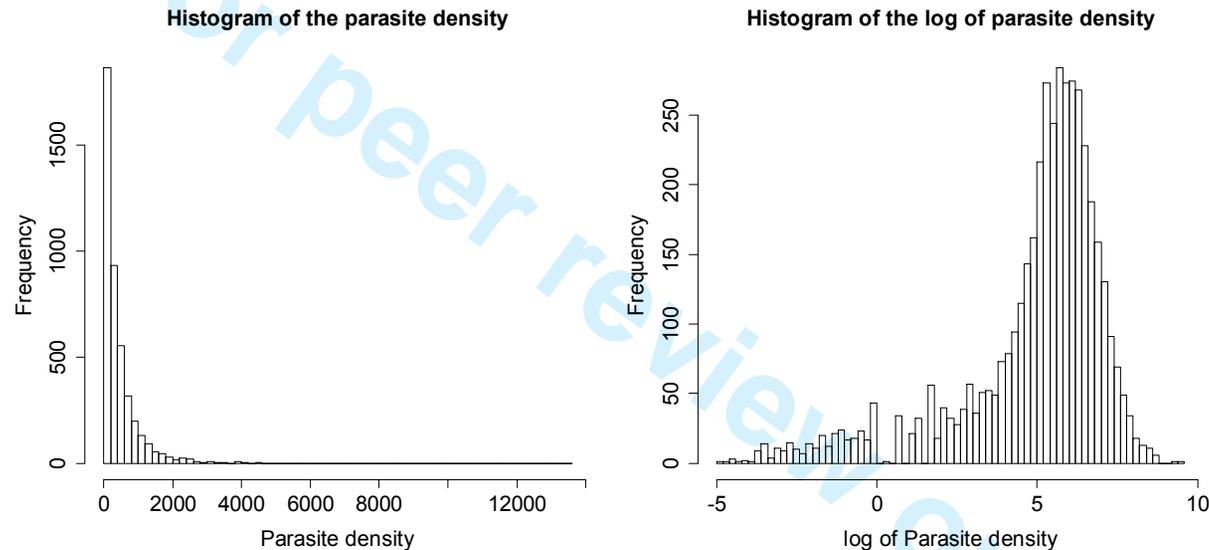
These figures provide a graphical checking of model goodness of fit. Figure A is the scatter plot of the natural logarithm of the observed parasite density and is compared to Figure B, which is the scatter plot of the natural logarithm of the predicted parasite density by the model; on both figures A and B the y-axes give the values for the log of the parasite density. Figure C shows the distribution of the residuals with the predicted values and Figure D is the histogram of the residuals; both figures C and D show the residuals normally distributed around zero.



Analysis using box-cox transformation and probit normalization

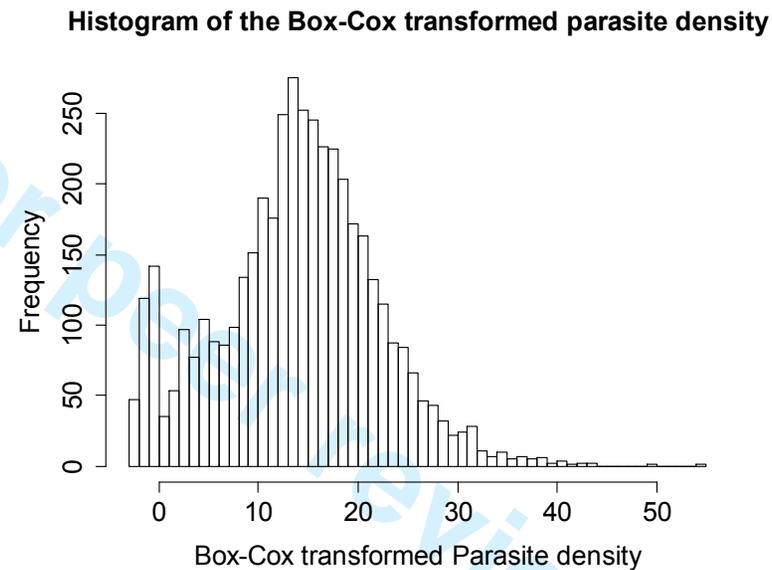
The model we fitted on the parasite density ("*pf_density*") has used as outcome variable the natural logarithm of *pf_density* (equivalent to a Box-Cox for which the parameter is null). As shown on Figure S4 the distribution of $\log(pf_density)$ is not perfectly normal, it is left-skewed.

Figure S4. Histogram of *pf_density* and $\log(pf_density)$



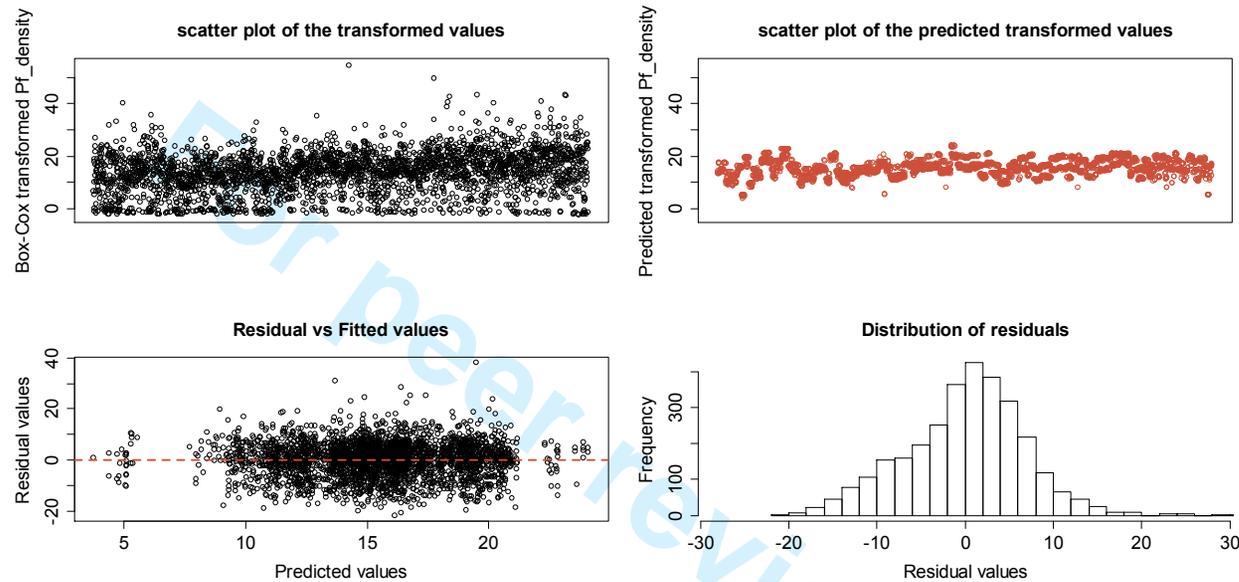
We add here the case for a Box-Cox transformation of the parasite density where the parameter is $\lambda = 0.3$, this parameter value was obtained as optimal using the R- function named "boxcox" from the "MASS" library. Then the Box-Cox transformation of the parasite density is $y = (pf_density^{0.3} - 1)/0.3$ having the distribution shown on Figure S5 below.

Figure S5. Histogram of the Box-Cox transformation of *pf_density* using a λ parameter of 0.3



With this Box-Cox transformed parasitemia as outcome variable, our results are maintained. Note that this distribution is not "perfectly" normal. However, the corresponding graphical control of the model adequation presented on Figure S6 below shows residuals more close to the normal distribution than those for $\log(pf_density)$ as outcome.

Figure S6. Graphical control of the model adequation for $y = \text{Box-Cox}(pf_density, \lambda = 0.3)$

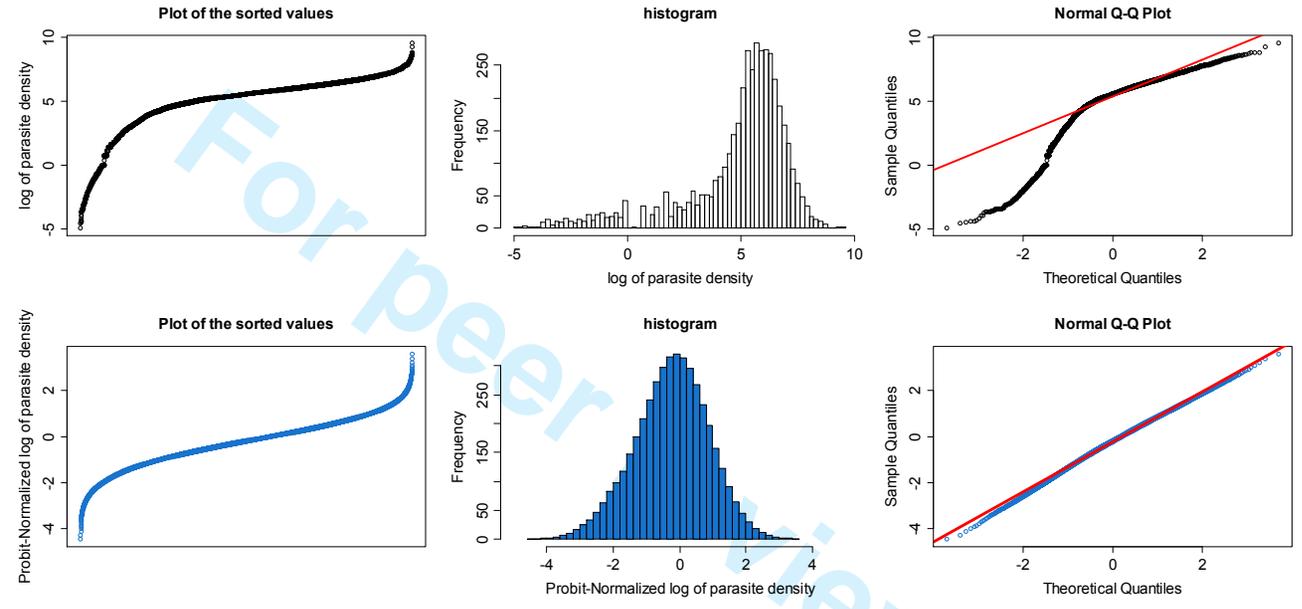


Although using a mixed model approach based on an extreme value distribution would provide a more robust validation of these results, the method we used incorporating pedigree information was developed through an R-package known as "pedigreemm" that allows just for a limited number of distribution laws, which do not include extreme value distributions like the Gumbel or Weibull distributions.

However, we tried the Probit normalization on the $\log(pf_density)$ to readjust its quantiles to those from a standard normal, and subsequently used the derived standard normal transformation of the $\log(pf_density)$ as outcome (see Figure S7 below, the three graphs presented in the first row of the graphs panel concern the $\log(pf_density)$ before Probit normalization and the three in the second row are for after Probit normalization. We can see on the histogram in blue color a good normal distribution of the y variable.

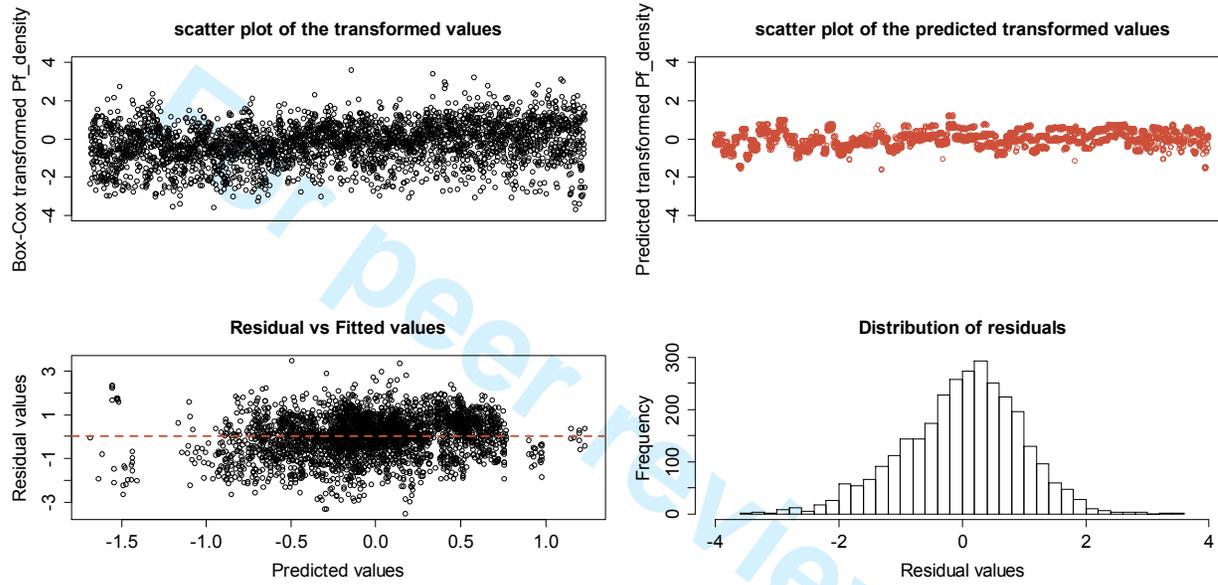
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Figure S7. Probit normalization of the $\log(pf_density)$



The results we obtained after this Probit normalization of the $\log(pf_density)$ confirmed the same findings. Also, the corresponding graphical control of the model adequation presented on Figure S8 below, shows a good normal distribution of residuals from this model.

Figure S8. Graphical control of the model adequation after Probit normalization of the $\log(pf_density)$



Asthma and atopic dermatitis are associated with increased risk of clinical *Plasmodium falciparum* malaria

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Article summary

Article focus

- Genetic studies suggest a link between susceptibility to allergy and malaria in Africa
- We hypothesize that atopy increases susceptibility to malaria

Key messages

- Results demonstrate an association between asthma, atopic dermatitis and susceptibility to clinical *P. falciparum* episodes.
- Genetic pre-disposition to asthma or atopic dermatitis impairs the acquisition of clinical immunity to malaria.
- Administration of anti-histamines to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Strengths and limitations

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association.

Abstract

Objectives: To assess the impact of atopy and allergy on the risk of clinical malaria.

Design: A clinical and immunological allergy cross-sectional survey in a birth cohort of 175 children from 1 month to 14 years of age followed for up to 15 years in a longitudinal open cohort study of malaria in Senegal. Malaria incidence data were available for 143 of these children (aged 4 months to 14 years of age) for up to 15 years. Mixed model regression analysis was used to determine the impact of allergy status on malaria incidence, adjusting for age, gender, sickle cell trait and force of infection.

Main outcome measures: Asthma, allergic rhinoconjunctivitis and atopic dermatitis status, the number of clinical *Plasmodium falciparum* malaria episodes since birth and associated parasite density.

Results: Twelve percent of the children were classified as asthmatic and ten percent as having atopic dermatitis. These groups had respectively a two-fold (OR 2.12 95% confidence intervals 1.46 to 3.08; $P=8 \times 10^{-5}$) and three-fold (OR 3.15, 1.56 to 6.33; $P=1.3 \times 10^{-3}$) increase in the risk of clinical *P. falciparum* malaria once older than the age of peak incidence of clinical malaria (3 to 4 years of age). They also presented with higher *P. falciparum* parasite densities (Asthma: mean 105.3 parasites/ μ L \pm SE 41.0 vs. 51.3 \pm 9.7; $P=6.2 \times 10^{-3}$; Atopic dermatitis: 135.4 \pm 70.7 vs. 52.3 \pm 11.0; $P=0.014$). There was no effect of allergy on the number of non-malaria clinical presentations. Individuals with allergic rhinoconjunctivitis did not have an increased risk of clinical malaria nor any difference in parasite densities.

Conclusion: These results demonstrate that asthma and atopic dermatitis delay the development of clinical immunity to *P. falciparum*. Despite the encouraging decrease in malaria incidence rates in Africa, a significant concern is the extent to which the increase in allergy will exacerbate the burden of malaria. Given the demonstrated anti-parasitic effect of anti-histamines, administration to atopic children will likely reduce the burden of clinical malaria in these children, increase the efficacy of first-line treatment anti-malarials and alleviate the non-infectious consequences of atopy.

Introduction

The World Allergy Organization estimates that 40% of the world's population is concerned by allergic diseases.¹ In developing countries where *Plasmodium falciparum* malaria is endemic, prevalence of allergy is significantly lower, but is on the increase.² T helper type 2 (Th2) cells, their related cytokines, IgE, eosinophils and mast cells play a major role in allergic inflammation. Orientation of the immune response towards a Th1 profile is crucial for immunity to intracellular pathogens,³ whereas orientation towards a Th2 profile drives immunity to extracellular pathogens and antigens resulting in class switching giving rise to IgE-producing B cells.⁴ ~~An important~~ role of the Th1/Th2 balance in the development of clinical malaria following infection by *P. falciparum* has been suggested by numerous studies.⁵⁻⁷ Whilst it is recognised that acquired anti-parasite immunity is IgG dependent,⁸ it has been suggested that the Th2 bias induced by *P. falciparum* may exacerbate allergy parasite-specific IgE also impact upon the clinical outcome of infection.⁸ For example, higher IgE but not IgG levels have been observed in patients with cerebral malaria than those with uncomplicated *P. falciparum* infection.⁹ The role of IgE, however, remains unclear.¹⁰ Likewise, an atopic state may generate a tendency to develop a Th2 type immune response to *P. falciparum*.

~~However,~~ the interplay between infectious agents and allergy is unclear/ambiguous. On the one hand, for example, severe respiratory syncytial virus infection in infants increased the risk of allergic rhinoconjunctivitis and allergic asthma.^{119,120} On the other hand, measles,¹³⁴ hepatitis A¹⁴² and tuberculosis¹⁵³ seemingly reduce atopy. Although, an atopic condition can increase incidence of disease, such as the case for the skin commensal *Staphylococcus aureus* in patients with atopic dermatitis,¹⁶⁴ an atopic tendency *per se* does not generally lead to increased illness from infectious agents.

Genome wide studies have identified chromosomal regions linked to clinical malaria, all of which overlap with those previously identified to be involved in atopic dermatitis, asthma, atopy and IgE levels,¹⁷⁵⁻¹⁹⁷ suggesting that common mechanisms may be involved in both pathologies.²⁰⁴⁸ Chromosomal region 5q31 that has been repeatedly shown to be associated with control of parasite density and contains a cluster of cytokines, among which IL12B has been previously associated with psoriasis.²¹⁴⁹ The other regions, 13q13-q22, 5p15-p13 and 12q21-q23, contain genes involved in innate immunity, notably the interleukin 7 receptor,

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and several involved in tumour necrosis factor synthesis [C1q and tumour necrosis factor related protein 3 (C1QTNF3)] and a gene involved in the complement system (C9).²⁰¹⁸

Several additional lines of evidence support the concept that susceptibility to malaria and atopy may be related to similar immunological defects. In Ethiopia, a history of malaria was associated with atopy.²²⁹ A mouse model for human atopic disease was found to be very susceptible to murine malaria and a major locus for atopic disease mapped close to the region controlling parasite density.²³¹ This region contains several candidate genes that have effects on T-cell function.²³¹

Moreover, a direct effect of histamine in the malaria pathogenesis has been found using genetic and pharmacological approaches²⁴² and increased levels of histamine are associated with the severity of disease in humans infected with *P. falciparum* and in animal malaria models.^{253,264}

To test the hypothesis that allergy impacts upon clinical *P. falciparum* malaria, we performed a clinical allergy cross-sectional study in the family-based longitudinal cohort from Senegal previously used for the genome linkage study²⁰¹⁸ and analysed the impact of asthma, atopic dermatitis, allergic rhinoconjunctivitis on the incidence of clinical *P. falciparum* episodes and the maximum parasite density during each episode.

Methods

Population and outcome data

The malaria research program conducted in Dielmo village in Senegal has been ongoing since 1990 as described elsewhere.²⁷⁵ In brief, between 1990 and 2008, a longitudinal study involving the inhabitants of the village of Dielmo, Senegal, was carried out to identify all episodes of fever. The study design included daily medical surveillance with systematic blood testing of individuals with fever and examination of 200 oil-immersion fields on a thick blood film for malaria parasites (about 0.5 µL of blood). Each individual was given a unique identification code and details of family ties, occupation, and precise place of residence were recorded on detailed maps of each household with the location of each bedroom. All households were visited daily, absenteeism recorded, and the presence of fever or other

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7 symptoms assessed. We systematically recorded body temperature at home three times a
8 week (every second day) in children younger than 5 years, and in older children and adults in
9 cases of suspected fever or fever-related symptoms. In cases of fever or other symptoms,
10 blood testing was done at the dispensary by finger prick, and we provided detailed medical
11 examination and specific treatment. Parasitologically confirmed clinical malaria episodes
12 were treated according to national guidelines. From 1990 to 2008, four different drug
13 regimens were implemented: Quinine from 1990 to 1994, Chloroquine from 1995 to 2003,
14 Fansidar (sulfadoxine-pyrimethamine) from 2004 to mid-2006 and Artemisinin-based
15 combination therapy (ACT; Amodiaquine- sulfadoxine-pyrimethamine) from mid-2006 to
16 2008.

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22 Parasite positivity was established as follows. Thick blood films were prepared and stained
23 by 3% Giemsa stain. Blood films were examined under an oil immersion objective at x1000
24 magnification by the trained laboratory technicians and 200 thick film fields were examined
25 to count the number of asexual and gametocyte parasite stages. Asexual parasite densities
26 (per μL) were calculated by establishing the ratio of parasites to white blood cells and then
27 multiplying the parasite count by 8,000, the average white blood cell count per μL of blood.

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32 Malaria transmission in Dielmo is intense and perennial. We conducted a cross-sectional
33 survey to estimate the prevalence of symptoms related to allergic diseases among 175
34 children aged from 1 month to 14 years old who were born during the malaria research
35 program.

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38 Both the longitudinal and cross-sectional surveys were approved by the Ministry of Health of
39 Senegal. Informed consent of the volunteers is renewed every year. More specifically for the
40 cross-sectional survey, after informing about the procedures and the purpose of the study,
41 written informed consent was obtained from parents or guardians of children either by
42 signature or by thumbprint on a voluntary consent form written in both French and Wolof,
43 the main local language. Consent was obtained in the presence of the school director, an
44 independent witness.

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49 The family structure (pedigree) was available after a demographic census performed for
50 every volunteer at his adhesion in the project. A verbal interview of mothers or key
51 representatives of the household was used to obtain information on genetic relationships
52 between studied individuals, their children, their parents, and to identify genetic links

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among the population. The total pedigree comprised 828 individuals, including absent or dead relatives, composed of ten independent families that can be sub-divided into 206 nuclear families (father – mother couples with at least one child) with an average of 3.6 children each. Genetically related nuclear families occur because of multiple marriages and marriages among related individuals. Previous typing with microsatellites has enabled the construction of a pedigree based on Identity-by-Descent using MERLIN.^{2048,286} The mean coefficient of inbreeding is 0.0008. Newborns since this original genetic analysis were added to the family of the parents in question. The 143 children, with both allergy and malaria data, belonged to 61 nuclear families and comprised 30 singletons, 102 siblings and 11 half-sibs (yielding 55 half-sib pairs). The mean genetic relatedness (by pedigree) of the 143 children is 0.0114 (range: 0.0013 to 0.022).

P. falciparum clinical episodes

P. falciparum malaria clinical episode phenotypes analysed were: (i) clinical *P. falciparum* infections treated with anti-malarial therapy and (ii) the highest parasite density during the *P. falciparum* clinical episode. A clinical *P. falciparum* episode was defined as a clinical presentation with fever (axillary temperature $\geq 37.5^{\circ}\text{C}$) and/or other clinical signs suggestive of malaria associated with a thick blood smear positive for *P. falciparum* and that was treated with anti-malarial therapy. Repeated clinical malaria presentations within 15 consecutive days were not considered to be independent and were excluded from the analyses, unless there was a negative thick blood smear between two clinical presentations. We also excluded observations in any trimester for which the individual was not present for at least one third of the time.

We calculated the quarterly incidence rate of clinical *P. falciparum* episodes in children below the age of 15 years as the ratio of the total number of clinical *P. falciparum* episodes during the trimester divided by the total number of person-trimesters surveyed. Incidence rate is expressed as cases per 100 person-trimesters (see Supplementary Figure S1). This rate was used in the analysis to approximate the force of infection (exposure level) within the targeted population at the time of a given clinical *P. falciparum* episode.

The total number of clinical presentations per trimester that were not attributable to *P. falciparum* was tabulated. Repeated non-malaria presentations within seven consecutive days were not considered to be independent and were excluded.

Allergic diseases and atopic status

The International Study of Asthma and Allergies in Childhood (ISAAC) diagnostic criteria have been shown to be reproducible, adequate and able to discriminate children with allergic diseases in different areas of the world.² The standardized ISAAC questionnaire originally written in English was translated into French in compliance with ISAAC guidelines²⁹⁷, adapting it to the usual local customs following advice from local clinicians and paediatric allergologists (Acknowledgements and Technical Appendix). The adequacy and reliability of the translated questionnaire had been previously confirmed by a pilot study on 30 randomly selected children in the same community. The questionnaire was completed by specially trained health workers during an oral interview conducted in Wolof with children and their mothers or guardians.

To assess the prevalence of allergic diseases in children, we used the positive and negative predictive values of the ISAAC questionnaire diagnosis criteria developed for subtropical countries.³⁰²⁸ Each question was scored according to the medical diagnosis of paediatricians and paediatric allergologists. Positive or negative answers were thus graded on the basis of symptom sensitivity, specificity, frequency, location or early onset. For each allergic disease, three categories of symptom severity, *severe*, *moderate*, and *none*, were defined as follows:

Asthma – *severe* symptoms if the child had “wheezing or whistling in the chest before the age of two years” and “more than three times” or severe enough to “limit his/her speech”; *moderate* symptoms if the child had “wheezing or whistling in the chest before the age of two years” and “in the past 12 months”; and *none* otherwise.

Allergic rhinoconjunctivitis – *severe* symptoms if the child had “sneezing, runny or stuffy nose in the past 12 months” and “more than five times a year”, and “itchy, watery eyes or tropical endemic limboconjunctivitis (TELC) in the past 12 months”; *moderate* symptoms if the child had “sneezing, runny or stuffy nose in the past 12 months”, and “itchy, watery eyes or TELC in the past 12 months”; and *none* otherwise.

Atopic dermatitis – *severe* symptoms if the child had “scaly or exudating, crusted and pruritic patches in the past 12 months” and “affecting any of the following characteristic areas: face, around the ears or eyes, folds of armpits or elbows or groin, behind the knees, under the buttocks”, and “onset of symptoms before the age of two years”; *moderate* symptoms if the

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7 child had “scaly or exudating, crusted and pruritic patches in the past 12 months” and
8 “affecting any of characteristic areas (see above)”, and “onset of symptoms before the age
9 of four years”; and *none* otherwise.

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11 The inter-relationships between variables reflecting the severity of symptoms of the three
12 allergic diseases were used to identify children at high risk of atopy. The *high probability*
13 group was defined by the prevalence of at least one of any *severe* symptoms or two of any
14 *moderate* symptoms. The *probable* group was defined as those with *moderate* symptoms
15 from one of the three allergic diseases and remaining children were classified in the *unlikely*
16 group.

21 *Helminths*

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23 Helminthic infections are common in this region and are known to modify the clinical course
24 and outcome of both allergic diseases and malaria.^{31,29,329} We therefore carried out a
25 helminth survey for 91 individuals present during the cross-sectional survey. Diagnosis was
26 performed by stool examination by microscope and by the Kato technique to search for the
27 presence of *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator*
28 *americanus*), whipworm (*Trichuris trichiuria*), *Schistosoma mansoni*, and *Strongyloides*
29 *stercoralis*. Examination for pinworms (*Enterobius vermicularis*) was performed by the anal
30 scotch-test. An anti-helminthic treatment was proposed for all infested individuals.

36 *Immunoglobulin E titres*

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38 Specific IgE titres were measured by ELISA as previously described.³³¹ A panel of allergens of
39 potential pertinence to the three classes of allergy was used: (i) Salivary gland extracts (SGE)
40 of two mosquito species present in the study cohorts, *Aedes aegypti* and *Anopheles gambiae*
41 *sensu stricto*, and (ii) *P. falciparum* parasite extract were prepared as previously described³¹;
42 (iii) House dust mite spp. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*;
43 (iv) a mix of pollen allergens from five ubiquitous gramineae spp. [Cock's-foot (*Dactylis*
44 *glomerata*), Timothy grass (*Phleum pratense*), Sweet Vernal grass (*Anthoxanthum*
45 *odoratum*), Perennial ryegrass (*Lolium perenne*), Kentucky Bluegrass (*Poa pratensis*)] (all
46 from Stallergenes, France).

52 **Statistical analysis**

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7 Statistical analyses were performed using R version 2.12.0 (The R Foundation for Statistical
8 Computing, Vienna, Austria). To address the effect of allergic status on the risk of clinical *P.*
9 *falciparum* episodes, we performed Generalized Linear Mixed Models (GLMM) extended to
10 pedigree data using the *pedigreemm* package for R to account for the non-independence of
11 individuals because of family relationships, shared house and for repeated measures from
12 the same individual (Technical Appendix). Correlated individual effects due to familial
13 relationships were taken into account by using the pedigree-based genetic relatedness
14 matrix that contains the genetic covariance among all pairs of individuals in the study cohort
15 and is calculated using the pedigree information.³⁴² Shared house and repeated measures
16 from the same individual were modelled as random effects. All random effects were
17 assumed to be normally distributed, and conditional on these random effects, the
18 dependent variable had: (i) a Binomial distribution when the studied phenotype was the
19 occurrence of a clinical *P. falciparum* episode treated with anti-malarial therapy during a
20 trimester, (ii) a Gaussian distribution when the studied phenotype was the logarithm of the
21 maximum parasite density during a given clinical *P. falciparum* episode, and (iii) a Poisson
22 distribution when the studied phenotype was the number of non-malaria episodes per
23 trimester. The effects of allergy disease classes on these dependent variables were modelled
24 as fixed effects. Allergy classes were reduced to two levels, *Severe* or *moderate* vs. *none* for
25 analyses of asthma, atopic dermatitis and allergic rhinoconjunctivitis and *high probability* vs.
26 *probable* and *unlikely* for atopic tendency. Co-variables included sickle cell trait³³¹, gender,
27 number of days present on site during the trimester, trimestrial incidence of *P. falciparum*
28 and age. Age was initially analysed as a continuous covariate. To assess the age-specific
29 effect of allergy, age was categorised into two levels (<3.5 years of age and ≥3.5 years of
30 age, based on the age of peak clinical incidence) and allergy class was nested within age
31 class. The age threshold was varied from 1.5 years to 5.5 years of age and the data re-
32 analysed to assess at which age there was the strongest effect. The association of allergy
33 classes with IgE levels was analysed by box-cox transforming the data and fitting a GLMM
34 with a normal distribution.
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51 Results

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Of the 205 eligible children aged under 15 years involved in the family-based longitudinal study, 175 (85.4 %) participated in the cross-sectional survey to assess the prevalence of related symptoms of allergic diseases. All eligible children present at the time of the survey were included; no explicit refusal to participate was recorded. The study cohort was aged from 1 month to 14 years 11 months. The sex-ratio (male/female) was 0.94.

From 1994 until 2008, 143 of the children participating in the cross-sectional survey were present for at least 31 days in any trimester during the study period generating a total of 3,093 person-trimesters of presence (Supplementary Table S1). There were 2,065 treated *P. falciparum* clinical episodes (per individual: median 11, range 0-47)(Supplementary Table S2). The age peak of incidence of *P. falciparum* episodes occurred at 3 to 4 years of age (Figure 1). There were 1,868 non-malaria episodes (median 12, range 0-37) (Table S2). These non-malaria clinical presentations were associated with headache (38 %), chills (32 %), cough (13 %), vomiting (11 %) and diarrhoea (6 %).

The prevalence of moderate or severe asthma symptoms was respectively 2.3 % and 10.3 % (Table 1). The prevalence of moderate or severe allergic rhinoconjunctivitis symptoms was respectively 6.3 % and 10.3 %. The prevalence of moderate or severe atopic dermatitis symptoms was respectively 6.3 % and 2.9 %. On the basis of symptom severity, an atopic tendency was estimated to be unlikely for 68.0 %, probable for 9.1 % and highly probable for 22.9 % of the 175 children. The frequency of each allergy class in children for whom malaria data were available is shown in Table S1.

The risk of treated clinical *P. falciparum* infections was higher for children with high probability of atopy (OR 1.65, 95% confidence intervals 1.20 to 2.26; P=0.002) (Table 2), after adjusting for age, sickle cell trait and the exposure level. Gender was not found to be significant. Analysing the impact of atopy in children younger and older than the peak age of clinical incidence (3 to 4 years old), revealed that atopy increased the risk of *P. falciparum* episodes in children at an age greater than 3.5 years (OR 2.02, 1.39 to 2.93; P=2x10⁻⁴), but not in children of age prior to the peak clinical incidence (OR 1.38, 0.92 to 2.08; P=0.124) (Table 2). This increased risk resulted in an ever increasing cumulative number of *P. falciparum* episodes with age beyond that of peak clinical incidence (Figure 2. See supplementary Figure S2 for model predictions for comparison).

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7 Analysis by allergy category revealed that asthma (severe or moderate) increases the risk of
8 *P. falciparum* episodes (OR 2.12, 1.46 to 3.08; $P= 8 \times 10^{-5}$) and this again only in children of
9 age greater than 3.5 years old (OR 2.33, 1.50 to 3.61; $P= 1.5 \times 10^{-4}$). Atopic dermatitis
10 increased the risk of clinical malaria in children older (OR 3.15, 1.56 to 6.33; $P= 1.3 \times 10^{-3}$) but
11 not younger than 3.5 years of age (Table 2). Allergic rhinoconjunctivitis was not associated
12 with increased risk of clinical malaria at any age (Table 2). The impact of atopy, asthma and
13 atopic dermatitis can be clearly seen in the ever-increasing number of cumulative *P.*
14 *falciparum* episodes beyond the age of the onset of clinical immunity in the population, 3.5
15 years of age (Figure 2). There is no difference in the number of clinical malaria episodes prior
16 to this age in individuals with or without an allergic condition. Analysis using different age
17 thresholds (from 1.5 to 5.5 years of age) revealed similar OR for thresholds of 2.5, 3.5 and
18 4.5 years of age. The maximum OR for increased malaria occurred in children older than 4.5
19 years of age and with atopy or atopic dermatitis, whereas for the asthma group it occurred
20 in children after 3.5 years of age (Supplementary Table S3).

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28 There was no impact of any allergic disease on the number of non-malaria episodes by
29 trimester (Supplementary Table S4).

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32 The impact of atopy, asthma and atopic dermatitis on the maximum *P. falciparum* parasite
33 density during a given clinical malaria episode mirrored that of the risk of *P. falciparum*
34 episodes. Parasite density was significantly higher for children with allergic disease older
35 than 3.5 years of age (Table 3 and supplementary Figure S3 for residuals of the fitted model).

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38 As the log-transformed data were left skewed, we additionally analysed using box-cox
39 transformation and probit normalization of the data. The results were qualitatively the same
40 (Supplementary text and Figures S4-S8). Allergic rhinoconjunctivitis had no impact on the
41 parasite density (Table 3). Analysis using different age thresholds yielded ~~the same~~
42 patterns similar qualitative conclusions as seen with the number of clinical episodes (Table
43 S3).

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48 Individuals with moderate or severe symptoms of atopic dermatitis had significantly higher
49 specific IgE titres against *Ae. aegypti* ($P=0.004$) and *An. gambiae* SGE ($P<0.001$). There were
50 no detectable specific anti-*P. falciparum* IgE. Individuals with moderate or severe symptoms
51 of allergic rhinoconjunctivitis did not have significantly higher IgE titres against the tested
52 gramineae ($P=0.28$), although titres decreased with age ($P=0.035$). There was also no effect of
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asthma on IgE titres against the house dust mite spp. tested (*D. farinae* P=0.60 & *D. pteronyssinus* P=0.27).

Only five individuals were infested with helminths (two *Ancylostoma*, one *Strongyloides*, one *Trichuris* and one *Enterobius*).

Discussion

Principal findings

Establishing the allergic status of children up to the age of 15 years old followed for malaria since birth, revealed an association of asthma and atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the increase in risk of malaria associated with these allergic conditions occurred after the peak clinical incidence of disease in the population, suggesting that they delay the development of clinical immunity to malaria.

Strengths and weaknesses of the study

The major strength of this study is the complete knowledge of the number of clinical *P. falciparum* malaria episodes each individual has had since birth and the exposure level per trimester over the 15 years covering the birth cohort. No other study has such detailed information for such a length of time. The major weakness of the study is the relatively small sample size, which would have reduced power to detect an association. In addition, although allergy diagnosis for children under 2 years of age is not considered reliable, there were only 15 individuals under 2 at the time of the allergy study of the 143 for whom malaria and allergy data were available.

Meaning of the study

Under intense malaria transmission, after repeated exposure to the parasite, children develop a clinical immunity³⁵³, whereby they tolerate elevated parasite densities without showing clinical symptoms. In this cohort, the population mean onset of clinical immunity occurred at 3 to 4 years of age. Although clinical immunity is accompanied by a reduction in parasite density, effective anti-parasite immunity develops much more slowly³⁶⁴ with individuals achieving a state of premunition, whereby they maintain low-grade parasite densities in an asymptomatic state.³⁷⁵ We show here that children with clinically defined

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7 | asthma or atopic dermatitis ~~had a two to three fold~~have an increased ~~in the~~ risk of
8 | presenting with *P. falciparum* malaria episodes requiring treatment once passing the age of
9 | peak clinical incidence. They also had higher parasite density during clinical episodes,
10 | suggesting a reduced ability to control parasite replication. The observed increase in clinical
11 | incidence of malaria in patients with asthma or atopic dermatitis is not likely to be the result
12 | of increased frailty of such individuals; these individuals did not come more frequently to the
13 | clinic with non-malaria symptoms. Our previous genome linkage study identifying
14 | chromosomal regions²⁰¹⁸ associated with malaria that overlap with those previously shown
15 | to be linked to asthma/atopy suggests that there may be a shared genetic basis to these
16 | pathologies rather than any causative effect of one on the other. This is consistent with the
17 | increased susceptibility to malaria of mouse atopic models.²³¹

23 | **Comparison with other studies**

25 | A previous study in Ethiopia (East Africa) found that a history of malaria (yes/no) increased
26 | risk of atopic dermatitis in 306 cases compared to 426 controls as characterized using the
27 | ISAAC questionnaire.²²⁹ The only other epidemiological study that has previously examined
28 | the link between malaria and atopy³⁸⁶ also interpreted the result from the perspective of the
29 | impact of malaria on atopy. They examined the re-infection rate with *P. falciparum* over a 5-
30 | year period in 91 children that were subsequently classified as atopic or not using skin prick
31 | tests (SPT) with house dust mite antigen. Their conclusion was that, as with measles¹³⁴ and
32 | tuberculosis¹⁵³, malaria infection reduces atopy. However, the study lacked previous
33 | infection data since birth of the participating individuals and focussed on atopy as
34 | determined by SPT against a single allergen. The case-control study of atopic dermatitis risk
35 | factors cited above found no overall association between allergen skin sensitization and
36 | atopic dermatitis. We also found no evidence of increased IgE titres against house dust mites
37 | in the asthmatic or atopic dermatitis groups or against grass pollen in individuals with
38 | allergic rhinoconjunctivitis. Such differences likely reflect the different IgE reactivity profiles
39 | due to differences in allergen exposure in Africa.³⁹⁷ There was no evidence of anti-parasite
40 | IgE in this cohort of children. We previously showed that circulating anti-parasite IgE titres
41 | were strongly positively correlated with anti-mosquito saliva IgE, but became undetectable
42 | following malaria exposure, potentially being bound to effector cells.³³¹ Only mosquito
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7 saliva, a known major local allergen, induced a specific IgE response at significantly higher
8 titres in individuals with atopic dermatitis.
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10 Although the immune effectors of clinical immunity are still poorly defined, there is strong
11 evidence that acquired anti-parasite immunity is IgG-dependent³⁸ and cytophilic
12 immunoglobulins (IgG1 & IgG3), which are capable of eliminating the parasites by
13 opsonisation and/or by Antibody Dependent Cellular Immunity play an important role in
14 premunition.³⁷⁵ The higher parasite density during symptomatic episodes observed in the
15 asthma group suggests impaired development of acquired immunity. Impaired acquisition of
16 immunity to malaria in children with asthma or atopic dermatitis may stem from their
17 imbalanced Th1/Th2 response. Indeed, an atopic state may generate a tendency to develop
18 a Th2 type immune response to *P. falciparum*. Dendritic cells that are oriented to a Th2
19 phenotype are more susceptible to orient the acquired immune response towards a Th2
20 profile.⁴⁰³⁹ Orientation of the immune response towards a Th2 profile by asthma or atopic
21 dermatitis would result in a poor Th1 response (and hence development of protective IgG
22 immunoglobulins), considered to be the dominant arm of the immune response enabling
23 resistance to infectious disease in children.⁴¹⁹
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32 Many studies have revealed an important role of histamine, a key downstream effector
33 molecule in allergic reaction, in the outcome of a malaria parasite infection.^{242-264,423-454}
34 Moreover, reports indicate that components of the innate immune system, including
35 eosinophils, basophils, and mast cells (MCs), could play important roles in the pathogenesis
36 of malaria.⁴²¹ Increased levels of histamine in plasma and tissue, derived from basophils and
37 MCs, notably following stimulation by IgE through the high affinity receptor FcεR1, are
38 associated with the severity of disease in humans infected with *P. falciparum* and in animal
39 malaria models.^{253,264} Chlorpheniramine, a HR1agonist reversed resistance to chloroquine
40 and amodiaquine both *in vivo* and *in vitro*.⁴³² Moreover, astemizole, another HR1 agonist,
41 was identified as an anti-malarial agent in a clinical drug library screen.⁴⁴³ Finally, *P.*
42 *falciparum* produces translationally controlled tumor protein, which is a homolog of the
43 mammalian histamine-releasing factor that causes histamine release from human
44 basophils.⁴⁵⁴
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52 Further research

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7 Our results provide the first birth cohort study addressing the link between malaria and
8 allergic diseases. They contribute to a growing body of evidence that the pathologies are
9 related. ISAAC has revealed a steady but significant increase in prevalence rates of asthma
10 and allergic diseases in Africa. Whilst the majority of studies have focused on large cities,
11 there is increasing urbanization throughout Africa, as well as improved access to primary
12 health care in many areas. A key concern for ISAAC is the extent to which such societal
13 evolution will result in an increase in allergic diseases. Increased urbanization in sub-Saharan
14 Africa is changing the epidemiology of malaria and although resulting in a decrease in risk,
15 will result in more severe clinical malaria in older individuals.^{465,476} Moreover, a large
16 consumption of anti-malarial drugs in the urban areas provides substantial drug pressure
17 fostering, the selection of drug-resistant parasites. Despite the encouraging recent decrease
18 in malaria incidence rates, even in rural areas, an additional significant concern is the extent
19 to which such an increase in allergy will exacerbate the burden of malaria. Given the
20 demonstrated anti-parasitic effect of anti-histamines^{47,48}, administration of anti-histamines
21 to atopic children will likely reduce the burden of clinical malaria in these children, increase
22 the efficacy of first-line treatment anti-malarials⁴⁹⁸ and alleviate the non-infectious
23 consequences of atopy. Clinical intervention studies should be envisaged.

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33 What is already known on this topic

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35 There are several reports of the beneficial effects of anti-histamines for malaria
36 chemoprophylaxis^{242-264,487} as well as our previous work²⁰¹⁸ showing that chromosomal
37 regions associated with malaria are also linked to allergy and atopy.¹⁷⁵⁻¹⁹⁷ There are two
38 epidemiological studies showing opposite effects of malaria on atopy.^{229,386}

41 What this study adds

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43 Using a longitudinal malaria study birth cohort, we identified an association of asthma and
44 atopic dermatitis with susceptibility to clinical *P. falciparum* episodes. Importantly the
45 increase in risk of malaria associated with these allergic conditions occurred only after the
46 peak clinical incidence of disease in the population, suggesting that they delay the
47 development of clinical immunity to malaria.

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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7 Ethical approval: The allergy study was approved by the Senegalese National Ethics
8 committee (2009/N°46). Renewed approval of the longitudinal malaria study was obtained
9 from the same committee (2006/N°969).
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11 Data sharing: The allergy database will be made available on-line. The longitudinal malaria
12 data set will be made available following discussion with the coordinators of the three
13 Institutes that govern the dataset through contact with the corresponding author.
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Table 1 Classification of Asthma, Allergic rhinoconjunctivitis, Atopic dermatitis and overall Atopic status according to ISAAC questionnaire in children aged 0-14 from a malaria birth cohort. N is total number of children examined and n-malaria represents those for whom malaria data were recorded. F is the number of females and M the number of males.

	N (F/M)	%	n-malaria (F/M)
Asthma symptoms			
None	153 (73/80)	87.43	125 (59/66)
Moderate	4 (1/3)	2.29	4 (1/3)
Severe	18 (6/12)	10.29	14 (4/10)
Rhinoconjunctivitis symptoms			
None	146 (64/82)	83.43	120 (52/68)
Moderate	11 (8/3)	6.29	9 (6/3)
Severe	18 (6/12)	10.29	14 (6/8)
Atopic dermatitis symptoms			
None	159 (75/84)	90.86	128 (60/68)
Moderate	11 (1/10)	6.29	11 (1/10)
Severe	5 (4/1)	2.86	4 (3/1)
Atopic tendency			
Unlikely	119 (56/63)	68.00	97 (46/51)
Probable	16 (8/8)	9.14	14 (6/8)
Highly probable	40 (16/24)	22.86	32 (12/20)

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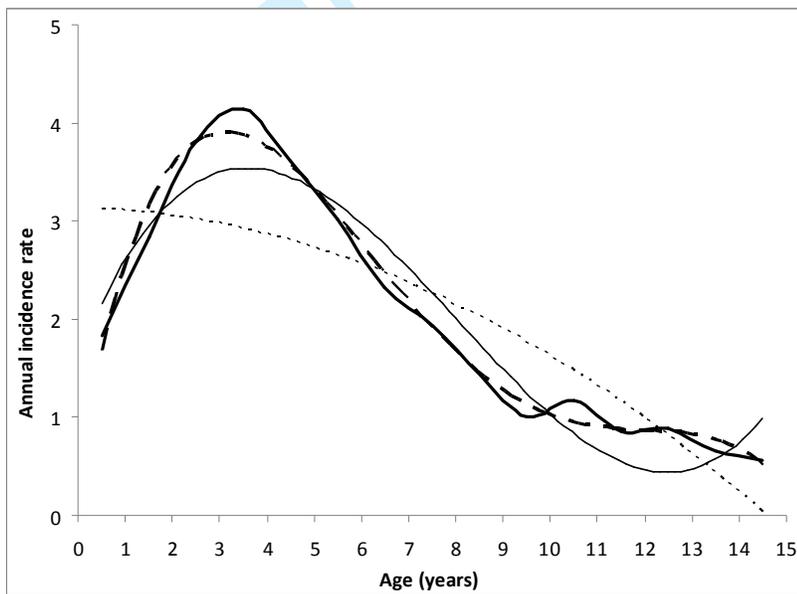
Table 2 Impact of allergy status on risk of *P. falciparum* clinical episodes. Shown are the *P* values and adjusted Odds Ratios with 95% confidence intervals calculated from the mixed model analyses. Values for the covariables Age (≥ 3.5 years of age compared to < 3.5 years of age), Trimestrial incidence of *P. falciparum* clinical episodes and HbAS (beta-globin sickle cell trait; AS compared to AA) are those from the Asthma model analysis. For clarity significant co-variables are shown in bold.

	Age groups < 3.5 years $>$	ORa	95% Confidence Intervals		<i>P</i> value
			Lower	Upper	
Atopy	Both	1.65	1.20	2.26	2.0×10^{-3}
	< 3.5	1.38	0.92	2.08	0.124
	≥ 3.5	2.02	1.39	2.93	2.1×10^{-4}
Asthma	Both	2.12	1.46	3.08	8.0×10^{-5}
	< 3.5	1.50	0.90	2.50	0.122
	≥ 3.5	2.33	1.50	3.61	1.5×10^{-4}
Atopic dermatitis	Both	1.05	0.65	1.70	0.842
	< 3.5	0.84	0.49	1.46	0.539
	≥ 3.5	3.15	1.56	6.33	1.3×10^{-3}
Rhinoconjunctivitis	Both	0.96	0.65	1.41	0.818
	< 3.5	1.05	0.64	1.72	0.853
	≥ 3.5	0.95	0.60	1.52	0.834
Age ≥ 3.5		0.48	0.40	0.57	2.7×10^{-15}
Trimestrial incidence		1.01	1.00	1.01	1.8×10^{-6}
HbAS		0.24	0.12	0.47	3.7×10^{-5}

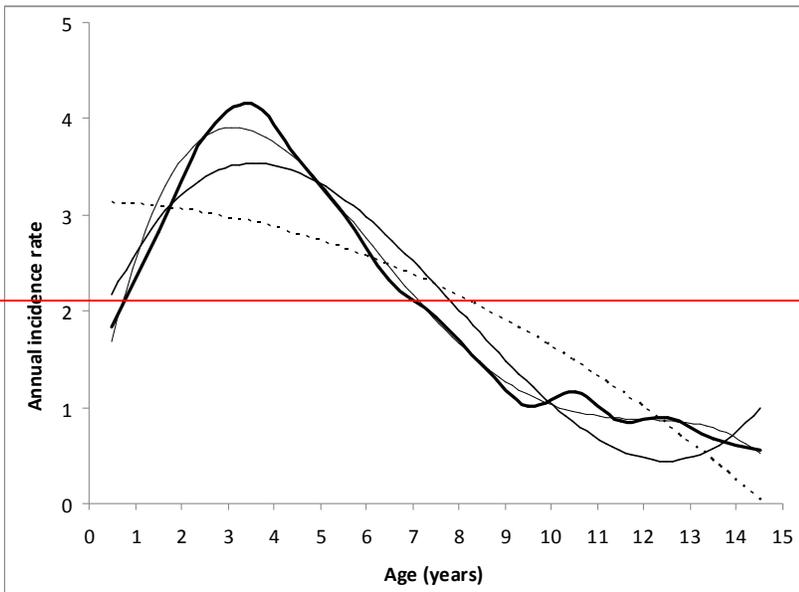
Table 3 Impact of allergy status on the maximum *P. falciparum* parasite density during a clinical malaria episode. Shown are the back-transformed mean parasite densities per microlitre and standard errors (SEM) estimated from the GLMM analyses after taking into account the other co-variables. Significantly different effects are shown in bold for clarity.

Allergic condition	Age groups	Allergic status (No/Yes)	Mean parasite density	SEM	P value
Atopy	Both	N	76.3	13.8	
		Y	131.0	36.4	0.0158
	<3.5	N	114.3	23.7	
		Y	171.1	56.0	0.148
	≥3.5	N	48.4	9.8	
		Y	114.8	37.1	9.5x10⁻⁴
Asthma	Both	N	78.1	14.4	
		Y	148.5	44.3	3.8 x10⁻³
	<3.5	N	114.8	24.3	
		Y	171.9	74.5	0.167
	≥3.5	N	51.3	9.7	
		Y	105.3	41.0	6.2 x10⁻³
Atopic dermatitis	Both	N	82.6	15.0	
		Y	93.9	38.9	0.605
	<3.5	N	122.6	25.5	
		Y	133.9	63.5	0.425
	≥3.5	N	52.3	11.0	
		Y	135.4	70.7	0.014
Rhinconjunctivitis	Both	N	81.5	14.8	
		Y	111.4	39.0	0.570
	<3.5	N	118.8	25.1	
		Y	166.3	69.9	0.537
	≥3.5	N	54.6	11.3	
		Y	80.9	33.7	0.327

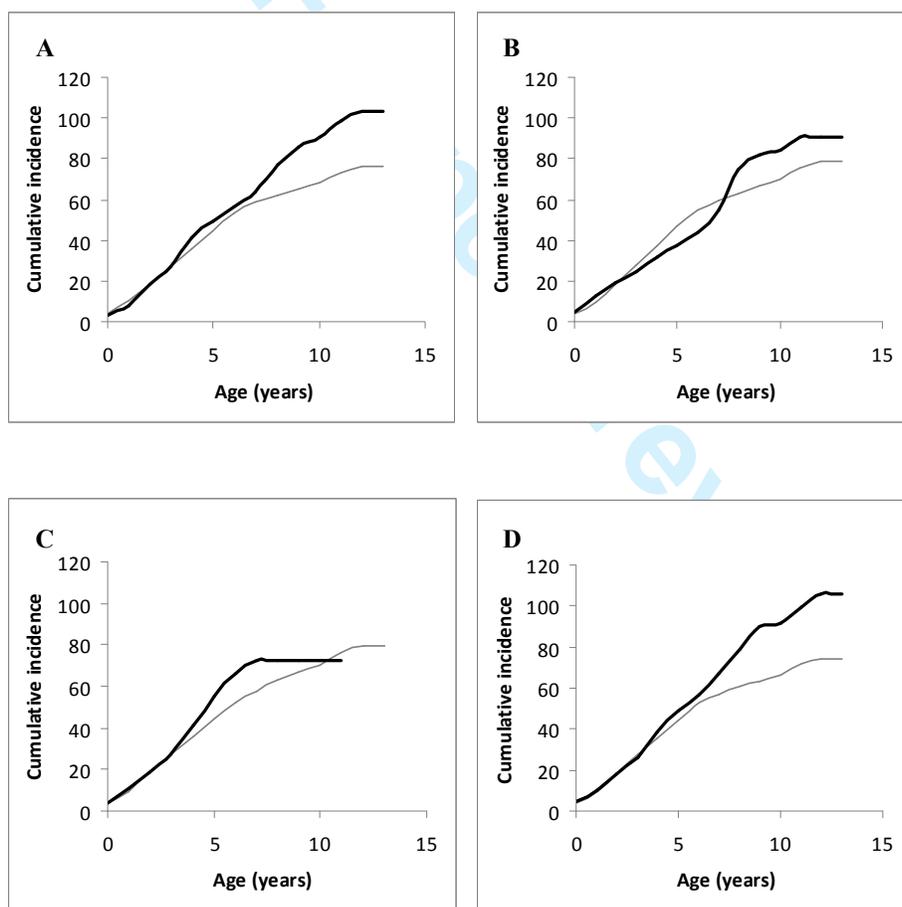
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7 **Figure 1** Annual incidence rate of clinical *P. falciparum* episodes per 100 children (bold
8 line). In order to overcome the fluctuations of the annual incidence rate, we fit second (dotted
9 line), third (dashed line) and fourth (solid line) degree polynomial trend lines to the data (bold
10 line). The corresponding R-squared values are 0.70, 0.91 and 0.99 respectively indicating an
11 accurate fit for third and fourth order polynomials. The inflexion on these two trend lines
12 indicates the onset of acquisition of clinical immunity at approximately 3 to 4 years of age.
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7 **Figure 2** Mean cumulative number of *P. falciparum* clinical episodes with age for the (A)
8 Asthma, (B) Rhinoconjunctivitis and (C) Atopic dermatitis classes and overall Atopy class
9 (D) (bold lines) compared to individuals without symptoms of each respective allergy type
10 (thin lines). In all cases moderate and severe classes are combined and compared to
11 individuals without allergy symptoms. Note there are no children older than 11 years of age
12 with Atopic dermatitis.
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3. Until what age did your child breastfeed **exclusively** without ever taking other aliments (fruits, vegetables, rice, meat, fish, etc.) or liquids (powdered milk, cow or goats milk, fruit juice, water, etc.) ?
 < 6 months ₁ 6 – 12 mths ₂ 12 – 24 mths ₃ NSP ₉

AGEBREAST

Illness and vaccination : Consultation of health records of child

1. Has your child enfant had the following illnesses?

Malaria : ₀ No ₁ Yes ₉ NSP

MALAR

Tuberculosis treated : ₀ No ₁ Yes ₉ NSP

TUBTRT

Helminths (oxyures, ascaris, taenia, etc.) : ₀ No ₁ Yes ₉ NSP

HEMINTH

Amoeba : ₀ No ₁ Yes ₉ NSP

AMOEBA

Measles : ₀ No ₁ Yes ₉ NSP

MEASLES

2. Against what illnesses is your child vaccinated?

Yellow fever : ₀ No ₁ Yes ₉ NSP

VACFJ

Hepatitis B : ₀ No ₁ Yes ₉ NSP

VACHEPB

Measles : ₀ No ₁ Yes ₉ NSP

VACMEASLE

Mumps : ₀ No ₁ Yes ₉ NSP

VACMUMPS

Rubella : ₀ No ₁ Yes ₉ NSP

VACRUBEL

Tuberculosis/BCG : ₀ No ₁ Yes ₉ NSP

VACTUB

Diphtheria/Tetanus/Pertussis/Poliomyelitis : ₀ No ₁ Yes ₉ NSP

VACDTCP

Typhoid : ₀ No ₁ Yes ₉ NSP

VACTY

Meningitis : ₀ No ₁ Yes ₉ NSP

VACMENIN

Haemophilus influenzae type B (HiB) : ₀ No ₁ Yes ₉ NSP

VACHIB

Habitation :

1. Which of these animals / insects can be found in the **rooms** where your child lives (today and/or during his first year of life) ?

Dogs in rooms today : ₀ No ₁ Yes ₉ NSP

DOGTODAY

Dogs in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

DOG01YR

Cats in rooms today : ₀ No ₁ Yes ₉ NSP

CATTODAY

Cats in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

CAT01YR

Sheep in rooms today : ₀ No ₁ Yes ₉ NSP

SHEEPTODAY

Sheep in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

SHEEP01YR

Goats in rooms today : ₀ No ₁ Yes ₉ NSP

GOATODAY

Goats in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

GOA01YR

Chicken, ducks in rooms today : ₀ No ₁ Yes ₉ NSP

CHICTODAY

Chicken, ducks in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

CHIC01YR

Rodents (rats, mice, etc.) in rooms today : ₀ No ₁ Yes ₉ NSP

RODTODAY

Rodents (rats, mice, etc.) in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

ROD01YR

Cockroaches in rooms today : ₀ No ₁ Yes ₉ NSP

COCTODAY

Cockroaches in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

COC01YR

Other in rooms today : ₀ No ₁ Yes ₉ NSP

OTHTODAY

Other in rooms 0-1yr : ₀ No ₁ Yes ₉ NSP

OTH01YR

If Others, define :

..... NAMEOTH

2. Which of these animals could be in **contact** with your child **at least once per week**

(today and/or during his first year of life) ?

Contact with Dogs today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CDOGTODAY
Contact with Dogs 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CDOG01YR
Contact with Cats today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CCATODAY
Contact with Cats 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CCAT01YR
Contact with Sheep today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CSHEEPTODAY
Contact with Sheep 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CSHEEP01YR
Contact with Goats today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CGOATODAY
Contact with Goats 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CGOA01YR
Contact with Chicken, Ducks today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CCHICTODAY
Contact with Chicken, Ducks 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CCHIC01YR
Contact with donkeys, horses today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHORSTODAY
Contact with donkeys, horses 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHORS01YR
Contact with Cows, zébus today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CCOWTODAY
Contact with Cows, zébus 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CCOW01YR
Contact with Rodents (rats, mice, etc.) today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CRODTODAY
Contact with Rodents (rats, mice, etc.) 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CROD01YR
Contact with Other today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	COTHTODAY
Contact with Other 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	COTH01YR
If Others, define :		<input type="checkbox"/>	NAMEOTHC

3. Which of these aliments are usually stocked in the rooms where your child lives ?

Millet kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MIL
Sorghum kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	SORG
Maize kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MAIZ
Rice kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RICE
Wheat kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WHEA
Biscuits, pasta kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	BISCUI
Manioc (root, flour) kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MANIOC
Cashew nut, ground nut kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	NUTP
Curdled milk kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MILKCURD
Dried leaves (mint, quinquiliba, baobab, etc.) :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	LEAF
Other aliments kept in room :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHALIM
If Others, define :		<input type="checkbox"/>	NAMEOTHAL

What is the type of roofing of the rooms where your child lives (today and during the first year of life) ?

Corrugated metal roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RMETTODAY
Corrugated metal roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RMET01YR
Thatched roof today:	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RTHATDAY
Thatched roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RTHAT01YR
Wooden roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RWOOTODAY
Wooden roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RWOO01YR
Cement roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RCEMTODAY
Cement roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RCEM01YR
Plaster roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RPLATODAY
Plaster roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RPLA01YR
Other type of roof today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ROTHTODAY

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Other type of roof 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ROTH01YR
If other, define :			NAMEOTHR
4. Which of these objects are in the room where your child sleeps (today and during the first year of life) ?			
Mattress in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MATR01YR
Mattress in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MATRTODAY
Bednet in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	BED01YR
Bednet in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	BEDNTODAY
Wardrobe in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WARD01YR
Wardrobe in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WARDTODAY
Chest, trunk in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHEST01YR
Chest, trunk in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHESTODAY
Table in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	TAB01YR
Table in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	TABPTODAY
Chair in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHA01YR
Chair in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHAPTODAY
Carpet, rug in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CARP01YR
Carpet, rug in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CARPTODAY
Matting in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MAT01YR
Matting in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	MATPTODAY
Curtains in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CURT01YR
Curtains in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CURTTODAY
Malagasy fire in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FIR01YR
Malagasy fire in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FIRTODAY
Other objects in room today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTH01YR
Other objects in room 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHOB01YR
If other, define :			NAMEOTHOB
5. On what type of bedding does your child sleep (today and during the first year of life) ?			
Foam mattress today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FMAT01YR
Foam mattress 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FMATRTODAY
Plant fibre mattress (straw, etc.) today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLFMAT01YR
Plant fibre mattress (straw, etc.) 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLFMATRTODAY
Wool mattress today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WOMAT01YR
Wool mattress 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	WOMATRTODAY
Feather mattress today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FEATHM01YR
Feather mattress 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	FEATHMTODAY
Plastic matting today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLMAT01YR
Plastic matting 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLMATRTODAY
Plant fibre matting (straw, etc.) today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLFMAT01YR
Plant fibre matting (straw, etc.) 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PLFMATRTODAY
Other type of bedding today :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHBED01YR
Other type of bedding 0-1yr :	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHBEDTODAY
If other, define :			NOMAUTLI
6. Does your child sleep on a pillow ?	<input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PILLOW
If No, go to question 8			

1	If Yes , what type of pillow is it ?		
2	Foam : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PILLF
3	Synthetic fibres: <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PILLSYN
4	Plant fibres (straw, etc.) : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PILLPLF
5	Feather : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PILLFEATH
6	Other type of pillow : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHPILL
7	If other, define :		NAMEOTHPILL
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11	7. Do people smoke in the room where your child lives ?		
12	Today : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	SMOKTODAY
13	From 0-1yr : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	SMOK01YR
14	During the pregnancy of the mother : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	SMOKPREG
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17	8. What type of heating and lighting are used in the rooms where your child lives ?		
18	Heating and lighting by charcoal : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHELCHAR
19	Heating and lighting by wood : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CHELWOO
20	Lighting by candle : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	LCAND
21	Lighting by petrol lamp : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	LLAMP
22	Lighting by flash light : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	LTORCH
23	Lighting by solar : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	LSOLAR
24	Other types of heating and lighting: <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHHEL
25	If other, define :		NAMEOTHHEL
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30	9. Which of the following products are used or stocked in the rooms where you child lives ?		
31	Insecticide (type Yotox, spirales, etc.) : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	INSECTIC
32	Deodorants (aerosols) : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	DEODORA
33	Incense : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	INCENSE
34	Detergents (type Cotel, etc.) : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	DETERGEN
35	Petrol, diesel : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	PETROL
36	Other types of products : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	OTHPROD
37	If other, define :		NAMEOTHPR
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41	Diet :		
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44	1. Has your child had diarrhoea without fever or abdominal pains (colic)		
45	following introduction of non-maternal milk in his diet (cow or goat's milk, milk powder) : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	DIARINT
46	after a few months of consuming non-maternal (cow or goat's milk, milk powder) : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	DIARMONTH
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50	2. Currently, how many times, on average, does your child eat the following aliments ?		
51	<i>The consumption of certain aliments is seasonal.</i>		
52	Meat : <input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSMEAT
53	Fish : <input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSFISH
54	Egg : <input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSEGG
55	Milk (liquid, powder, curdled) : <input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSMILK
56	Banana : <input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSBANA
57	Mango : <input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSMANG
58	Melon : <input type="checkbox"/> Never <input type="checkbox"/> <1times/week <input type="checkbox"/> 1-2 times/week <input type="checkbox"/> ≥1times/day	<input type="checkbox"/>	CONSMELON
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1	Orange, lime :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSORAN
2	Potatoes, sweet potatoes :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSPOT
3	Vegetables :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSVEG
4	Millet :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSMIL
5	Sorghum :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSSORG
6	Maize :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSMSAIS
7	Rice :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSRICE
8	Wheat (bread, pasta) :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSWHEA
9	Nuts (Cashew, ground nut) :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSNUT
10	Prawns, dried oysters :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSPRAWN
11	Flavouring cubes Maggi :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	CONSCUBE
12	Other :	<input type="checkbox"/> ₁ Never	<input type="checkbox"/> ₂ <1times/week	<input type="checkbox"/> ₃ 1-2 times/week	<input type="checkbox"/> ₄ ≥1times/day	<input type="checkbox"/>	OTHALCON
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If other, define :

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HISTORICAL SYMPTOMATOLOGY OF ALLERGIC REACTIONS

Asthma :

1. Has a doctor or nurse **already** said that you child has asthma ?

₀ No ₁ Yes ₉ NSP

ASTHMA

2. Has your child already breathed noisily or had whistling in his chest whilst breathing

₀ No ₁ Yes ₉ NSP

WHISTLING

If **No**, go directly to question 6

3. During his first two years of life, has your child already breathed noisily or had whistling in his chest whilst breathing ?

₀ No ₁ Yes ₉ NSP

WHISTL2YR

If **No**, go directly to question 6

If **Yes**, how many times (before 2 years of age) ?

₁ 1time ₂ 2times ₃ ≥3times ₉ NSP

NBWHIS2YR

Between the last two **ramadans**, has your child already breathed noisily or had whistling in his chest whilst breathing ?

₀ No ₁ Yes ₉ NSP

WHISTL2RA

If **No**, go directly to question 5

If **Yes**, at which moment of the year ?

Rainy season : ₀ No ₁ Yes ₉ NSP

WHISTLRS

Dry season : ₀ No ₁ Yes ₉ NSP

WHISTLDS

Harvest time : ₀ No ₁ Yes ₉ NSP

WHISTLHT

Has the noisy breathing of your child been such that it has prevented him from talking normally?

₀ No ₁ Yes ₉ NSP

PREVTALK

Has your child already had a rasping cough at night that prevents him from sleeping normally ?

₀ No ₁ Yes ₉ NSP

TOUSECHE

Rhinitis and allergic conjunctivitis:

1. Has your child **already had** problems of a runny nose, or a sensation of a blocked nose, or an itchy nose, or sneezing, or loss of the sense of smell **for more than a week**,

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irrespective of the frequency of these episodes? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHIN1WEEK
2. Has your child already had problems of a runny nose, or a sensation of a blocked nose, or an itchy nose, or sneezing, or loss of the sense of smell more than 5 times in one year , irrespective of the frequency of these episodes? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHIN5FAN
Between the last two ramadans , has your child already had problems of a runny nose, or a sensation of a blocked nose, or an itchy nose, or sneezing, or loss of the sense of smell ? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHIN2RAM
If No , go to question 4		
If Yes , at what moment of the year ?		
Rainy season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHINRS
Dry season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHINDS
Harvest time : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	RHINHT
3. Has your child already had watery eyes, or itchy eyes, or an allergic limbo-conjunctivitis? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJALER
If No , go directly to question 1 in the section Eczema		
Has your child had, between the last two ramadans , watery eyes, or itchy eyes, or an allergic limbo-conjunctivitis? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJ2RAM
If No , go directly to question 5		
If Yes , at what moment of the year ?		
Rainy season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJRS
Dry season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJDS
Harvest time : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	CONJHT
<u>Eczéma :</u>		
Has your child already had skin problems with dry patches or seeping cracked patches and itching ? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEMA
If No , the questionnaire has finished.		
Between the last two ramadans , has your child had skin problems with dry patches or seeping cracked patches and itching ?? <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZE2RAM
If No , go directly to question 3		
If Yes , at what moment of the year ?		
Rainy season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEMARS
Dry season : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEMADS
Harvest time : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEMAHT
1. Have these skin problems affected different parts of the body of your child ?		
Scalp : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZESCALP
Face : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEFAC
Around the eyes and ears : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	
Armpits : <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> NSP	<input type="checkbox"/>	ECZEEYEAR

STROBE Statement—checklist of items included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses

Continued on next page

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure <i>Cross-sectional study</i> —Report numbers of outcome events or summary measures
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

Discussion

Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based
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*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.